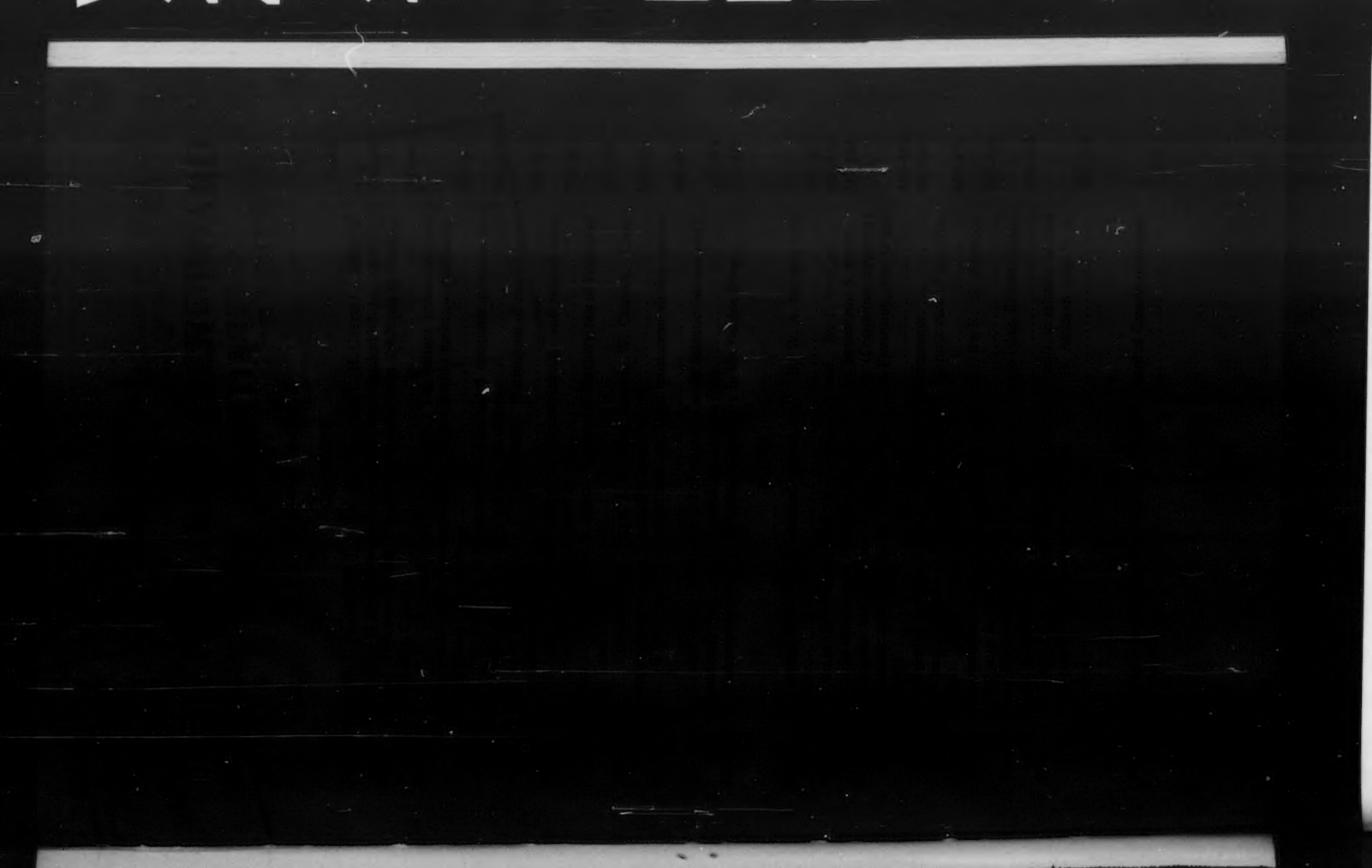

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Effect of Pasteurizing Doses of Gamma Radiation on Storage Life of Cooked Lobster Meat[1]

By H. E. Power, D. I. Fraser, W. J. Dyer, W. Neal, and C. H. Castell

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

ABSTRACT

Irradiation of cooked lobster (*Homarus americanus*) meat with a dose of gamma radiation of 75,000 rads extended the storage life, in ice, to between 28 and 35 days for both tail and claw meats from 14 to 21 days for unirradiated iced samples. Higher irradiation levels caused an immediate loss of quality due to toughening and loss of flavour. The growth of trimethylamine-producing bacteria was inhibited 10 days longer on irradiated samples than on the unirradiated iced control.

INTRODUCTION

Spoilage of marine products during iced storage is mainly due to growth of bacteria on the surface of the product. The fewer the spoilage bacteria present on the surface, the longer the useful storage life. For example, fillets cut from fresh cod under aseptic conditions and stored at 38 F (3.4 C) in sterile containers have been held for up to 40 days without changes in colour or odour (Castell, 1948).

Unfortunately, it is not possible to process marine products under commercial conditions whereby the processed material is free from spoilage bacteria on the surface of the animal. During processing, these spoilage bacteria are transferred to the processing equipment and thence to other products. Castell (1948) has shown that each 10-fold reduction in the number of bacteria contaminating a fillet during processing adds approximately 1 day to its storage life.

The bacteria on the finished product can be destroyed after packaging by the use of ionizing radiation (Nickerson et al., 1954). However, doses large enough to ensure sterilization have been found to cause undesirable changes in the taste, odour, and other characteristics of the product.

Substerilization doses of ionizing radiation have been shown by Masurovsky et al. (1963), Power et al. (1964a, 1964b), Brooke and Steinberg (1964), and others to extend the storage life of clams, haddock fillets, scallops, and other marine products to several times their normal storage life in ice by lowering the initial bacterial contamination. Since the effects of ionizing radiation on the appearance, odour, and taste of marine products vary widely with species and dose (Rhodes, 1964), the characteristics and optimum dose

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for each species must be determined. The present study describes the effect of pasteurization doses of gamma radiation on the organoleptic characteristics and storage life of cooked lobster meat stored at 0 C.

EQUIPMENT AND METHODS

Cobalt 60 Source

The irradiation facility used for the treatment of samples in this experiment was the Gammacell 220 designed and constructed by Atomic Energy of Canada Ltd. (Rice and Smythe, 1960), as described previously (Power et al., 1964a).

Bacterial Counts

Bacterial plate counts were made on blended mixtures of claw and tail meat with a nutrient agar described by Dyer et al. (1946). The counts were made after the plates had been incubated for 72 hr at 25 C.

Thiobarbituric Acid Value

The thiobarbituric acid value, a measure of oxidative rancidity, was determined by the distillation procedure of Tarladgis et al. (1960).

Trimethylamine Analysis

As a measure of extent of bacterial spoilage the trimethylamine values of blended mixtures of claw and tail meat were determined as described by Dyer (1945, 1959).

Organoleptic Assessment

In preparation for tasting, the cooked, iced meats in plastic bags were brought to a serving temperature of 10–15 C by placing the bags into water at 25 C for approximately 10 min. Frozen control lobster meats were allowed to thaw overnight in a refrigerator. In addition, freshly boiled and cooled meats prepared from lobster obtained live from a local seafood market were used as "known" controls at each tasting. As in the taste-testing of irradiated scallops and of irradiated haddock (Power et al., 1964a, 1964b), the lobster meats were graded for taste, texture, and overall acceptability by an expert panel of tasters; characteristics of appearance and odour were also noted. Taste panel procedure was similar to that previously described (Power et al., 1964b). Claw and tail meats were assessed separately. In a freshly boiled lobster these differ appreciably in texture; the claw meat is soft, tender, and only slightly fibrous, whereas the tail meat is much more fibrous and chewy. The claw meat has been shown to deteriorate more rapidly on frozen storage (Bligh et al., 1957).

A series of preliminary panels, using samples both of cooked, iced, non-irradiated control meats and of meats irradiated at pasteurizing doses ranging

from 25,000 to 300,000 rads, was run to determine the irradiation levels suitable for use in the present study and to establish the progression of changes in flavour and texture characteristics during iced storage up to the point of unacceptability. A level of 300,000 rads proved excessive, resulting in burned off-odours and flavours, some yellowing of the tail meats, and a reduction in score to the barely acceptable level. On the other hand, the relatively low dose of 25,000 rads did not prolong the storage life in ice beyond the 28 days' keeping time observed in the unirradiated meats. From these observations, irradiation levels of 75,000, 150,000, and 250,000 rads were chosen for the principal study, and a suitable score sheet for organoleptic assessment was devised (Table I).

EXPERIMENTAL

The lobsters used were caught in traps off Yarmouth, Nova Scotia. They were transported by truck to a commercial lobster processing plant, where they were held alive in wooden tanks in running sea water for 2 weeks. Their average weight was 570 g.

At the processing plant, the lobsters were cooked for 20 min in water containing 6.6% salt, the standard commercial procedure used in preparation of lobster meat for the restaurant trade. They were then shucked on the regular processing line, and the meats rinsed in fresh water. The meats were weighed, packed, and heat-sealed in laminated cellophane–polyethylene bags. Each bag contained the claw and tail meats from one lobster.

The bags were immediately packed in ice and transported to the laboratory, where they were divided into five groups. One group was frozen in a pre-chilled plate freezer (Dole Freze-Cel model 2735-6; plate temperature −38 C) and stored at −26 C for use as a frozen control for the taste panel studies. A second group was packed in ice to serve as an iced control (unirradiated). The remaining three groups were irradiated with 75,000, 150,000, and 250,000 rads respectively, at a dose rate of 15,150 rads per min, and subsequently stored in ice. All samples were treated and stored within 8 hr of cooking.

At intervals of 1 week, samples were removed from storage for organoleptic, chemical, and bacteriological evaluation.

RESULTS

Bacterial Examination

The initial bacterial count of the iced control samples was 6900 colonies per g lobster meat (Table II). Irradiation at a dose level of 75,000 rads reduced the count to 120, at 150,000 and 250,000 rads to less than 100.

After 7 days storage in ice at 0 C, the bacterial count per gram of the iced control samples had reached 129,000 whereas the count for all irradiated samples was less than 100. The count on the iced control sample increased steadily to 510×10^6 at 21 days.

TABLE I. Score sheet used in the taste testing of control and irradiated lobster meat.

Name of Judge: Date:

Taste	1 CT[a]	2 CT	3 CT	4 CT	5 CT	6 CT
0 Sweet, meaty, fresh, seawater taste						
1 Some loss of sweetness more or less tasteless						
2 Neutral						
3 Some off-flavours (acid-sweetish, veg.–H_2O, faint irradiation)						
4 Definite off-flavours seaweedy aftertaste						
5 Objectionably sour, NH_3 fruity, musty, bitter						

Remarks:

Texture	1 CT	2 CT	3 CT	4 CT	5 CT	6 CT
0 Moist, firm, rather fibrous (typical lobster)						
1 Tougher, chewier						
2 Some softness, sponginess some dryness, mealiness						
3 Definite softness, sponginess definite dryness, mealiness						
4 Tough, stringy very dry						
5 Rubbery hard inelastic						

Remarks:

Odour and Appearance: Remarks

1.
2.
3.
4.
5.
6.

Opinion	1 CT	2 CT	3 CT	4 CT	5 CT	6 CT
0 Perfect						
1 Good						
2 Fair						
3 Borderline						
4 Slightly spoiled						
5 Spoiled						

[a]C, claw meat; T, tail meat.

TABLE II. Bacterial counts per gram of control and irradiated lobster meats after storage in ice.

Days in storage	Iced control	Frozen control[a]	Irradiated		
			75,000 rads	150,000 rads	250,000 rads
1	6,900	7,500	120	<100	<100
7	129,000	6,000	<100	<100	<100
14	45×10^6	6,300	240,000	126,000	78,000
21	510×10^6	5,100	90×10^6	3.3×10^6	2.1×10^6
28	–	8,700	660×10^6	219×10^6	204×10^6
35	–	8,200	$1{,}000 \times 10^6$	600×10^6	66×10^6
42	–	2,100	315×10^6	551×10^6	171×10^6

[a]Stored at –26 C.

Bacterial growth on the irradiated samples was slower, reaching counts of 90×10^6, 3.3×10^6, and 2.1×10^6 at 21 days for samples treated with doses of 75,000, 150,000, and 250,000 rads respectively, and counts of 1000×10^6, 600×10^6, and 66×10^6 after 35 days.

The frozen control samples had bacterial counts between 2100 and 8700 per g over the storage period.

TRIMETHYLAMINE VALUES

The TMA value of the iced control meat increased rapidly after 20 days storage to a level greater than 30 mg N/100 g (Fig. 1). The TMA values for the irradiated samples remained below 10 mg N/100 g for 30 days but then increased rapidly. Irradiation with 75,000–250,000 rads thus inhibited the growth of TMA-producing bacteria for 10 days longer than on iced controls. The TMA value of the frozen control remained below 2 mg N/100 g throughout the storage period.

THIOBARBITURIC ACID VALUES

The TBA values were low (0.02 to 0.10) for all samples, indicating no increase in lipid oxidation during the 42-day iced storage period.

ORGANOLEPTIC ASSESSMENT

TASTE

Taste scores of claw and tail meats of the cooked, iced control, and irradiated lobster meats used in the principal study are shown in Fig. 2. The iced controls, both claw and tail meats were judged unacceptable[2] at 21 days

[2]With the numerical scoring system used (Dyer and Dyer, 1949), ranging from 100 (perfect) to 0 (very badly spoiled), a score of 40 or lower denotes unacceptability.

C, claw meat; T, tail meat.

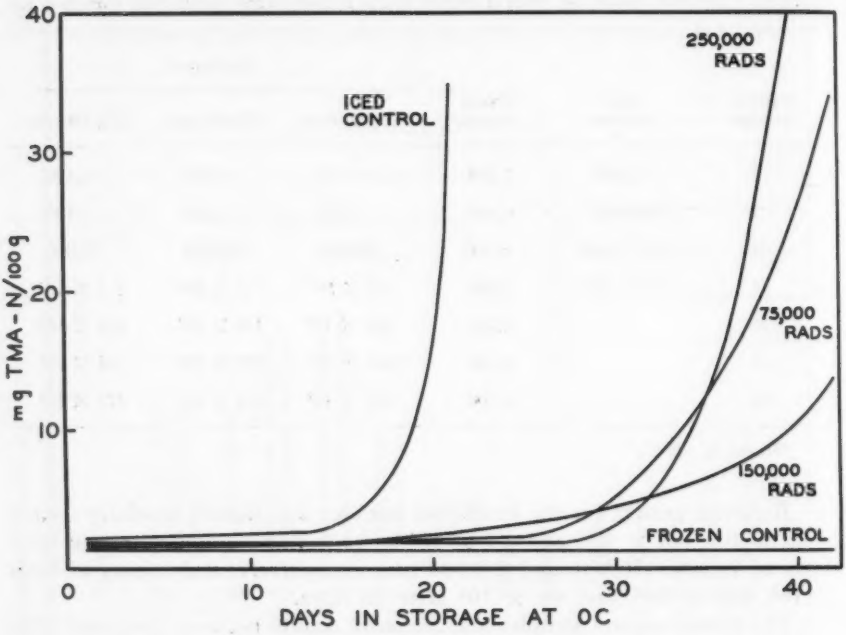

FIG. 1. Trimethylamine values for irradiated and control samples of cooked lobster meat stored in ice for various periods. The frozen control sample was stored at −26 C.

when strong spoilage odors, described as milky, fruity, and yeasty, were evident. The shorter keeping time of these iced controls compared with the 28 days found for the laboratory-processed controls in the preliminary study was undoubtedly because of heavier bacterial contamination encountered in the commercial processing.

The frozen control meats deteriorated slightly over the 42-day storage period due to a loss of the sweet, meaty, seawater flavour characteristic of the freshly boiled meats which were rated consistently high at an average level of 94.

The meats irradiated at 75,000 rads did not differ initially from the iced control meats; scores decreased gradually, as a result of developing acid-sweetish, seaweedy, and stale flavours, to the still acceptable level of 60 at 28 days, followed by a rapid drop to complete unacceptability at 35 days. Irradiation at 150,000 and 250,000 rads reduced the initial taste scores to approximately 60 and 50 respectively due primarily to a loss of the sweet, fresh flavour and to the presence of slight irradiation odours and flavours, especially in the samples irradiated at 250,000 rads. The meats irradiated at 150,000 were judged to be unacceptable at 35 days. The samples irradiated

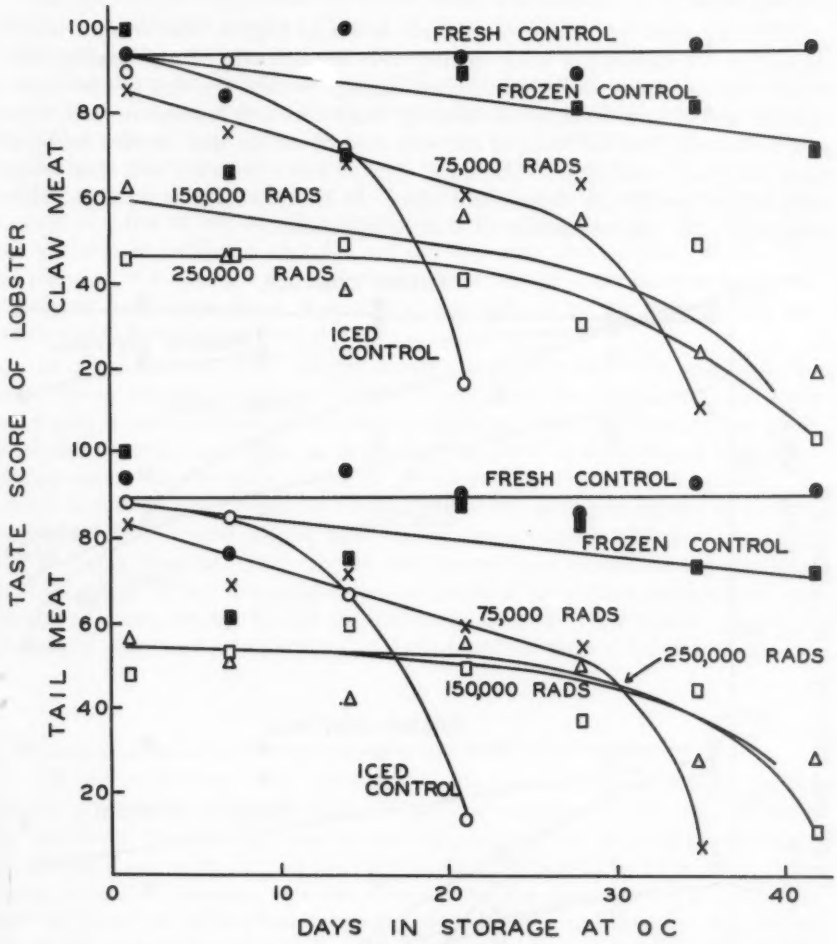

FIG. 2. Taste scores for irradiated and control samples of cooked lobster claw and tail meats stored in ice for various periods. The frozen control sample was stored at −26 C.

at 250,000 rads remained near the borderline level of acceptability for 35 days, then deteriorated rapidly to inedibility at 42 days.

In all samples of control and irradiated meats no significant differences in flavour scores were observed between the claw and tail meats. For all meats, the times at which flavour scores dropped to unacceptable levels corresponded closely with those times at which rapid production of TMA occurred.

TEXTURE

The changes in texture scores are shown in Fig. 3. The freshly boiled control meats assessed at each tasting were consistently of top quality. The frozen control meats were downgraded slightly toward the end of the experimental period as a result of developing toughness and dryness in both claw and tail meats. The tail meat of the iced control sample had become rubbery, hard, and unacceptable at 21 days, the time at which both tail and claw meats were judged inedible on flavour and odour. At 21 days the texture of the claw meat was near the borderline level of acceptability.

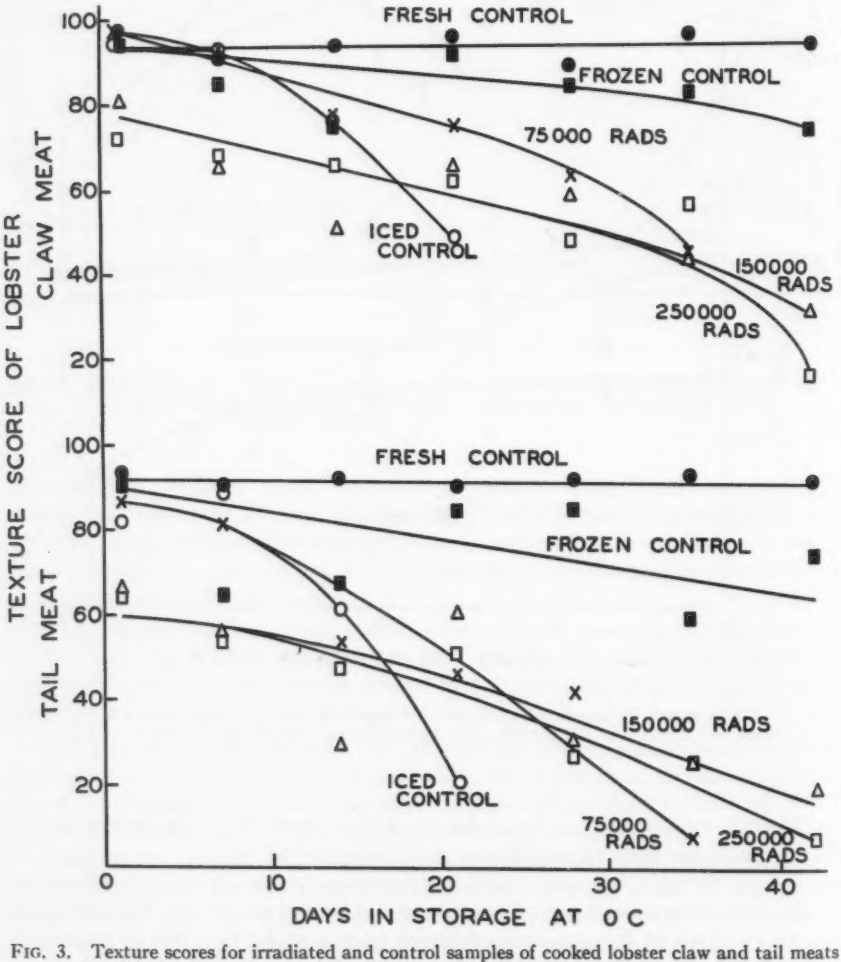

FIG. 3. Texture scores for irradiated and control samples of cooked lobster claw and tail meats stored in ice for various periods. The frozen control sample was stored at −26 C.

In the samples irradiated at 75,000 rads, the texture of the tail meats was at the borderline level at 21 and 28 days; in the claw meats, chewiness, "shortness," and dryness did not develop to the borderline level until 35 days. The doses of 150,000 and 250,000 rads imparted a slight toughness, especially to the tail meats, which lowered the initial texture scores to about 60; tail meats in both samples had become unacceptably tough and hard at 28 days, whereas claw meats were not judged unacceptable in texture until 42 days. A slight yellowing of the tail meat was apparent from the beginning in a few of the samples irradiated at the higher levels, but yellowing was variable in incidence and did not develop with storage time in ice. The red pigment of both claw and tail meats, especially of those samples receiving the higher irradiation doses, was observed to rub off on the fingers after approximately 4 weeks of iced storage.

In all samples of iced lobster meats, both claw and tail portions, the deteriorative changes in texture which accompanied the flavour changes were invariably a development of toughness, dryness, and chewiness, rather than of mushiness and gumminess as previously observed in control and irradiated scallop meats in the later stages of deterioration (Power et al., 1964a). A similar toughening has been reported as one of the principal factors in quality deterioration of cooked lobster meat during frozen storage (Bligh et al., 1957; Murphy and Newson, 1963). In the iced control and irradiated samples used in the present study, the toughness and dryness were more pronounced and developed more quickly in the tail meats than in the claw meats; the latter frequently exhibited a "shortness" of texture rather than a definite toughness.

DISCUSSION

Of the irradiation levels investigated for commercially processed cooked lobster meat in the present study, 75,000 rads appears to be the optimum. At this level, the keeping time at ice temperature was extended to between 28 and 35 days for both claw and tail meats from the 14 to 21 days for unirradiated meats. This dose was also found to be the optimum for scallop meat and for haddock fillets (Power et al., 1964a, 1964b), and doubled the storage life in ice. As with haddock and scallops the higher doses of 150,000 and 250,000 rads reduced initial scores of cooked lobster meats to a level between "fair" and "borderline" where they remained for over 30 days.

Few data on the effects of pasteurizing doses of irradiation on cooked lobster meat are available in the literature. At the Torry Research Station (1961, p. 13–14) a level of 100,000 rads had no detectable effect on flavour of cooked lobster meats and extended the keeping time at ice temperature to one-half to three times that of untreated controls. A review by Proctor et al. (1960) indicates a level of 250,000 rads for boiled lobster claw and tail meats as the maximum dose for acceptance with a storage life of 166 days at 35 F (2 C) for both 250,000 and 500,000 rads.

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Two New Species of Calanoid Copepods from the Strait of Georgia, British Columbia, Canada[1]

By Tai Soo Park;[2]

Friday Harbor Laboratories
University of Washington
Friday Harbor, Washington

ABSTRACT

Two new species, *Gaidius columbiae* and *Tharybis fultoni*, from the Strait of Georgia, British Columbia, Canada, are described and illustrated in detail. The former can be distinguished from all previously known species of its genus by the shape of the postero-lateral process of the metasome, and the latter by the shape of the 5th pair of legs.

INTRODUCTION

The two new species of calanoid copepods described below were found during examination of plankton samples collected from the Strait of Georgia, B.C., Canada, by the Pacific Oceanographic Group, Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C., and kindly placed at my disposal by Mr John Fulton of that Group.

The plankton copepods of the Strait of Georgia were studied by McMurrich (1916), Wailes (1929), Campbell (1929, 1930), and Légaré (1957). The last listed all the species that had been reported from the area. Recently, Park (1966) made known a new calanoid copepod, *Bradyidius saanichi*, among the local fauna.

To correspond with the prevailing literature the anatomical terms used in the descriptions below are defined as follows: metasome — the tagma composed of those thoracic segments normally bearing swimming legs; prosome — the cephalosome and metasome, thus the portion of the body anterior to the major articulation.

GAIDIUS COLUMBIAE, NEW SPECIES (FIG. 1–3)

Localities and Materials: Numerous specimens in plankton samples collected in the Strait of Georgia in 1965.

Types: Holotype female (total length = 3.00 mm), U.S. National Museum No. 113464; allotype male (total length = 2.90 mm), U.S.N.M. No. 113465; 5 paratypes of each sex, U.S.N.M. No. 113466.

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[1]Received for publication July 14, 1966.
[2]Present address: Woods Hole Oceanographic Institution, Woods Hole, Massachusetts.

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FEMALE

The total length from the anterior end of the cephalosome to the end of the caudal ramus ranges between 2.97 and 3.20 mm according to 30 randomly selected specimens. The prosome is about 3.5 times the length of the urosome. The 1st metasomal segment (to which the 1st swimming legs are attached) is fused with the cephalosome, leaving no trace of articulation (Fig. 1A, B). The 4th and 5th metasomal segments are likewise fused, carrying at the posterolateral corner a conical process of varying size and shape (Fig. 1C–I). The forehead is produced ventrally into a rather small rostrum, which is bifurcated at the tip (Fig. 1J). The urosome consists of 4 segments and caudal rami that are about as long as wide and carry a short external seta, a slightly curved ventral seta, and 4 subequal terminal setae.

The antennules reach the end of the genital segment when folded back and consist of 24 free segments, for segments 8 and 9 are fused (Fig. 1K). The antennae (Fig. 1L) have a 2-segmented endopod and a 7-segmented exopod, the latter being about 1.5 times as long as the former. The 2nd exopodal segment has on the internal margin a characteristic conical process tipped with a seta. The mandible (Fig. 1M) bears a strong masticatory blade armed with about 8 teeth and a basal seta. Three rows of spinules are found at the base of the 5 teeth next to the basal seta. In the mandibular palp the basis has 2 long setae, and the 1st endopodal segment 2 minute setae.

The maxillule (Fig. 1N) has a strong gnathobase bearing 14 strong spines along the internal margin and a few spinules at the base. The 2nd and 3rd inner lobes are about equal in size and each carries 4 setae. The maxilla (Fig. 2A) consists of 5 prominent lobes and a rather small endopod that bears 6 long and 1 short setae. The 2nd, 3rd, and 4th lobes have on each a round patch of spinules, and one of the three spines on the 4th and 5th lobes is markedly stouter than the others. The maxilliped (Fig. 2B, C) has a well-developed basipod, of which the coxa bears 4 lobes armed with 1, 2, 3, and 3 setae respectively. In addition, the last lobe bears a small triangular process pointed distally. The coxa has no lamelliform processes as found in some species of the genus.

In the first leg (Fig. 2D) the endopod is 1-segmented, but the exopod is 2-segmented. The 1st segment of the latter, however, consists of 2 fused segments, bearing a line of demarcation clearly visible in stained specimens, and has an internal seta and an external spine that is about one-third the length of the corresponding spine on the 2nd segment. The 2nd leg (Fig. 2E) has a 1-segmented endopod and a 3-segmented exopod. The former, however, consists of 2 fused segments as evidenced by a line of demarcation partially visible in stained specimens. The 3rd (Fig. 2F) and 4th (Fig. 2G, H) legs are similar; both the endopod and the exopod are 3-segmented. However, they are different in details of the coxa, which, in the 3rd leg, carries along the internal margin a row of hairs but, in the 4th leg, a row of 10–12 tubular structures.

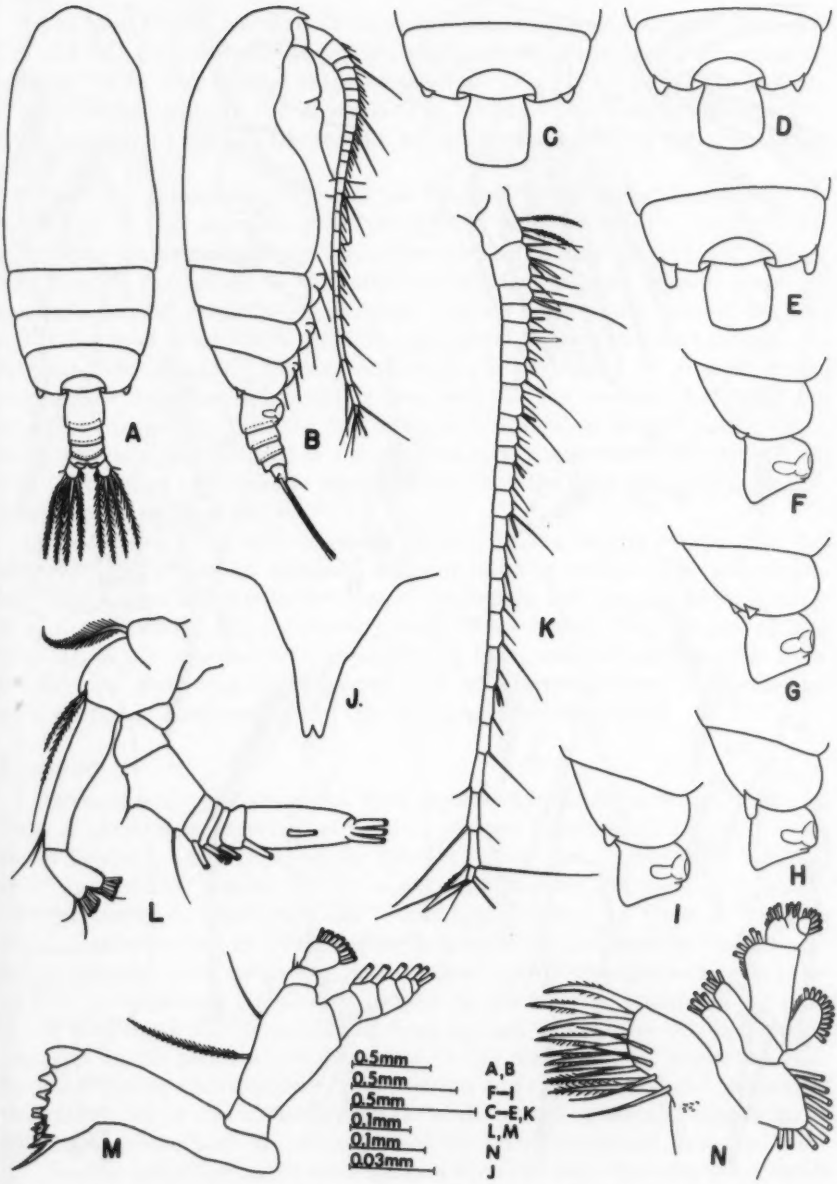

FIG. 1. *Gaidius columbiae*, new species. Female: A, habitus, dorsal view; B, habitus, lateral view; C–E, posterior part of metasome, dorsal view; F–I, posterior part of metasome, lateral view; J, rostrum; K, antennule; L, antenna; M, mandible; N, maxillule.

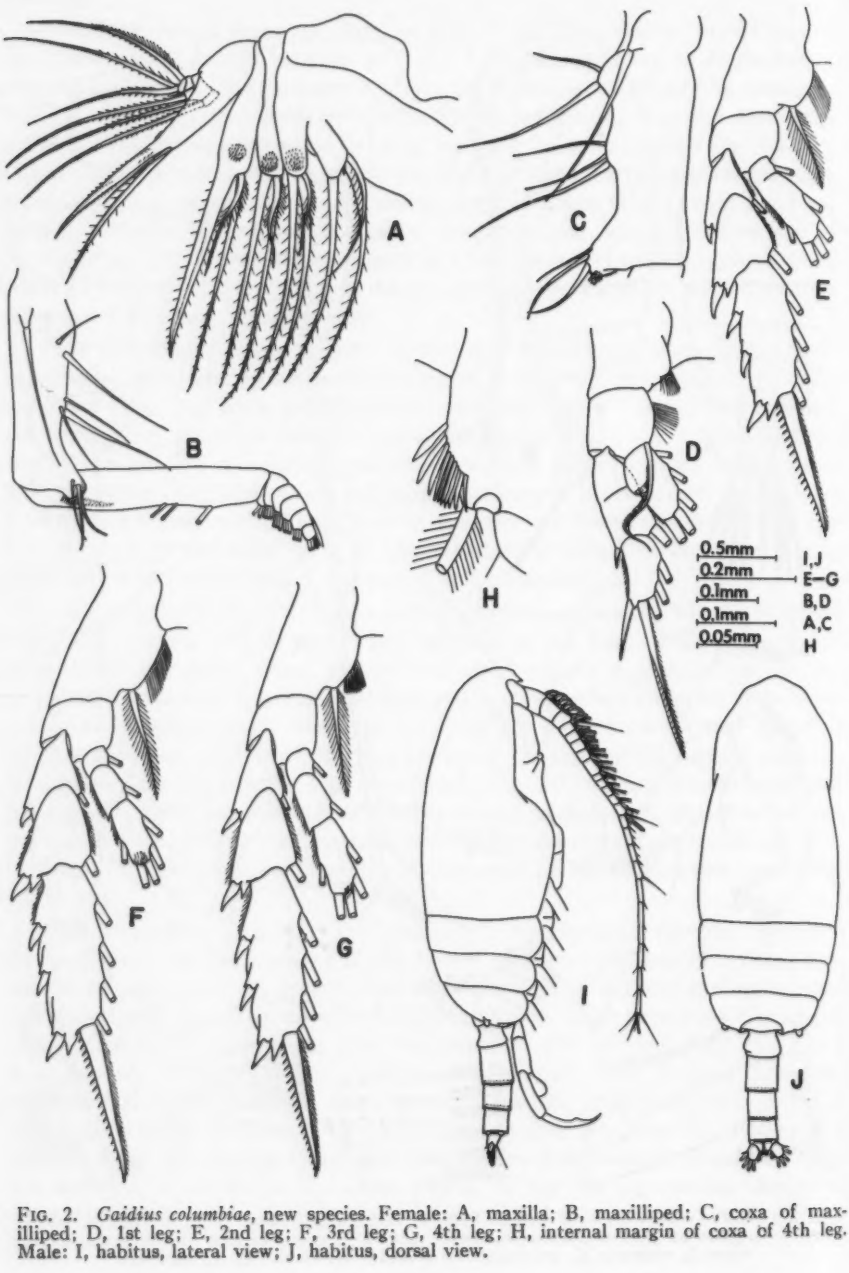

FIG. 2. *Gaidius columbiae*, new species. Female: A, maxilla; B, maxilliped; C, coxa of maxilliped; D, 1st leg; E, 2nd leg; F, 3rd leg; G, 4th leg; H, internal margin of coxa of 4th leg. Male: I, habitus, lateral view; J, habitus, dorsal view.

MALE

The total length, based on 20 randomly selected specimens, varies between 2.72 and 3.10 mm, and the ratio of the lengths of the prosome and the urosome is about 73:27. The general shape of the body (Fig. 21, J) is similar to that of the female, although the urosome (Fig. 3A) is 5-segmented. The rostrum is also similar to that of the female, being forked at the tip into two small points (Fig. 3B).

The two antennules (Fig. 3C) are identical. They differ from those of the female in that segments 8–10, 20–21, and 24–25 are fused, although the fusion between segments 9 and 10 is incomplete. The antenna (Fig. 3D) differs from that of the female in the following details: the coxa bears a patch of fine hairs, the 2nd exopodal segment has 2 regular setae on the internal margin, and the exopod is relatively short, being slightly longer than the endopod. In the mandible (Fig. 3E) the masticatory blade is reduced to a small single process, the basis has only a single seta, and the 2nd endopodal segment no setae. The maxillule (Fig. 3F) has no setae or spines on the 1st to 3rd inner lobes. The maxilla is reduced to a small process that lacks setae. The maxilliped (Fig. 3G) differs from that of the female in that the coxa bears only 2 setae plus a small process distally.

The 1st leg (Fig. 3H) is similar to that of the female except that the external spine of the 1st exopodal segment is much smaller. The 2nd to 4th legs (Fig. 31) are also similar to those of the female, but the coxa of the 4th leg lacks the row of tubular structures present in the female. The 5th pair of legs (Fig. 3J, K) is asymmetrical, the right leg being substantially heavier than the left one. Both legs are biramose, each with a 1-segmented endopod. The right exopod is 2-segmented and the left exopod is 3-segmented.

REMARKS

Seven species of *Gaidius* have been reported from the North Pacific Ocean. Four of them were reported as new: *G. pungens* Giesbrecht, 1895; *G. brevirostris* Brodsky, 1950; *G. variabilis* Brodsky, 1950; and *G. moderatus* Tanaka, 1957. In addition, Esterly (1911) reported *G. tenuispinus* Sars, and Wilson (1950) reported *G. affinis* Sars and *G. brevispinus* (Sars). Of these, *G. pungens* and *G. tenuispinus* are characterized by long spiniform processes on the postero-lateral corners of the metasome, and *G. affinis* and *G. brevispinus* by the presence of a conspicuous lamelliform process on the external margin of the coxa of the maxilliped. Of the remaining three species, which have relatively short processes on the postero-lateral margins of the metasome, *G. brevirostris* may be recognized by the relatively small rostrum and the last exopodal segment of the left 5th leg of the male. *Gaidius variabilis* and *G. moderatus* appear to be the same species; both are characterized by small metasomal processes.

Gaidius columbiae can be distinguished from not only those species reported from the North Pacific Ocean but also all the species in the genus by the characteristic shape of the process at the postero-lateral corner of the metasome. *Gaidius columbiae* is most similar to *G. variabilis* Brodsky of all known species

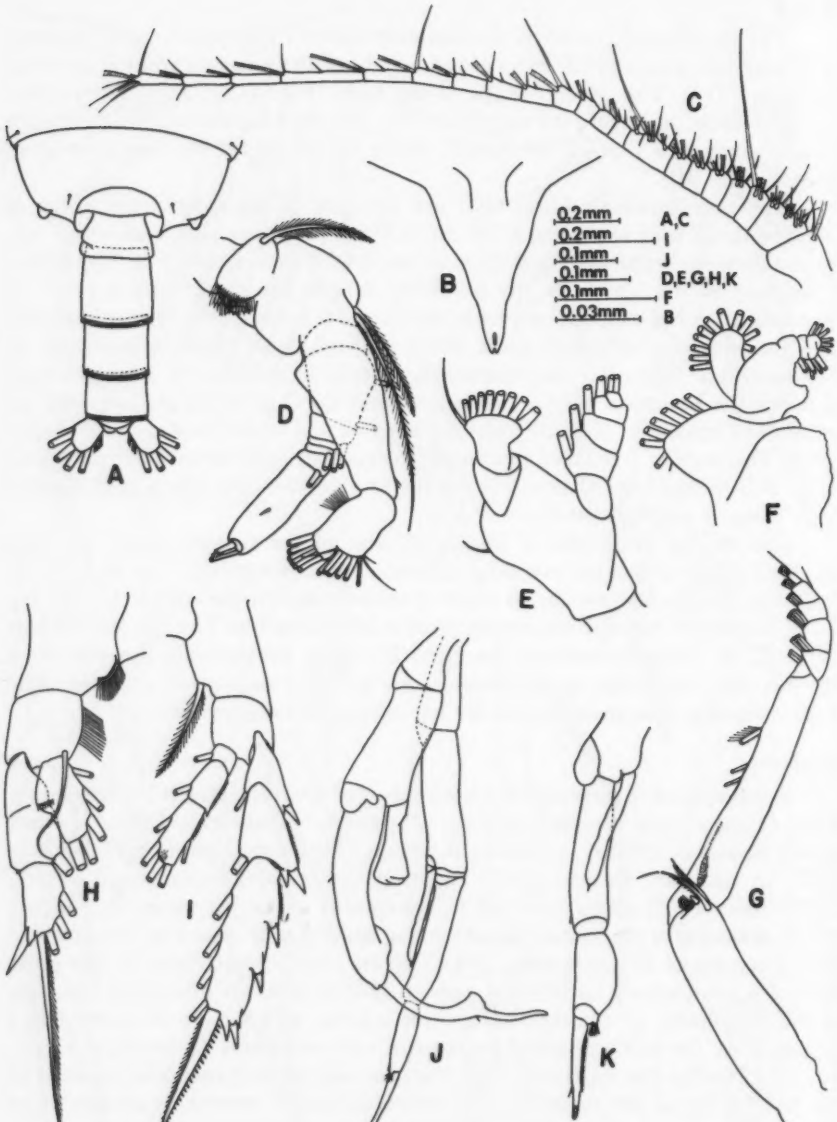

FIG. 3. *Gaidius columbiae*, new species. Male: A, posterior part of body; B, rostrum; C, antennule; D, antenna; E, mandibular palp; F, maxillule; G, maxilliped; H, 1st leg; I, 4th leg; J, 5th pair of legs, anterior aspect; K, distal part of left 5th leg.

of the genus, the only detectable anatomical difference being the size and shape of the metasomal processes. This characteristic, however, seems to be highly variable in both species, but within their own ranges.

Tanaka (1957) reported *G. minutus* Sars from Japanese waters, but his identification is presumed to be incorrect, for his descriptions and figures are not in full agreement with those of Sars (1925). Tanaka's specimens, however, seem to belong to *G. columbiae*, although they differ, according to his descriptions and figures, from the latter in the following minor respects: the antennule reaches to the distal end of the urosome rather than to the end of the genital segment, the rostrum is shorter, and the external spines on the 1st and 2nd legs are relatively larger than in *G. columbiae*.

THARYBIS FULTONI, NEW SPECIES (FIG. 4, 5)

Localities and Materials: Twenty-five females and 3 males in plankton samples collected from deep water close to the bottom in the Strait of Georgia in January and March, 1966.

Types: Holotype female (total length = 1.30 mm), U.S. National Museum No. 113467; allotype male (total length = 1.15 mm), U.S.N.M. No. 113468; 10 female paratypes, U.S.N.M. No. 113469.

Female

The total length based on 25 specimens ranges from 1.25 to 1.31 mm, and the ratio of the lengths of the prosome and the urosome is about 77:23. The body (Fig. 4A, B) is ovoid and bears a short urosome. The 1st metasomal segment is fused with the cephalosome and the 5th metasomal segment with the 4th. The postero-lateral corner of the metasome is produced distally into a narrow angle, as seen from the dorsal side. Seen laterally, these corners are broadly rounded, reaching to the end of the genital segment. The rostrum (Fig. 4C) consists of two slender filaments borne on a low round process. The urosome is 4-segmented; only the 2nd and 3rd segments are fringed distally with a row of fine spinules. The caudal rami are slightly longer than wide and bear a small dorsal spine, a ventral seta, and 4 terminal setae. The 2nd terminal seta from inside is about twice as long as the other 3 subequal setae.

The antennules reach to the end of the 3rd metasomal segment when held backward against the body and have 24 free segments as segments 8 and 9 are fused (Fig. 4D). The antenna (Fig. 4E) is characterized by the exceptionally long exopod, which is more than twice as long as the endopod. The mandible (Fig. 4F) has a strong masticatory blade, of which the cutting edge bears stout setiform teeth proximally and molariform teeth distally. The setiform teeth are thickly covered by spines. In the mandibular palp the basis with 2 spiniform processes and a long seta is fused with the 1st endopodal segment, which has 2 setae.

The maxillule (Fig. 4G) has an exceptionally large gnathobase, which is armed with 14 spines and extends as far as the end of the rest of the appendage.

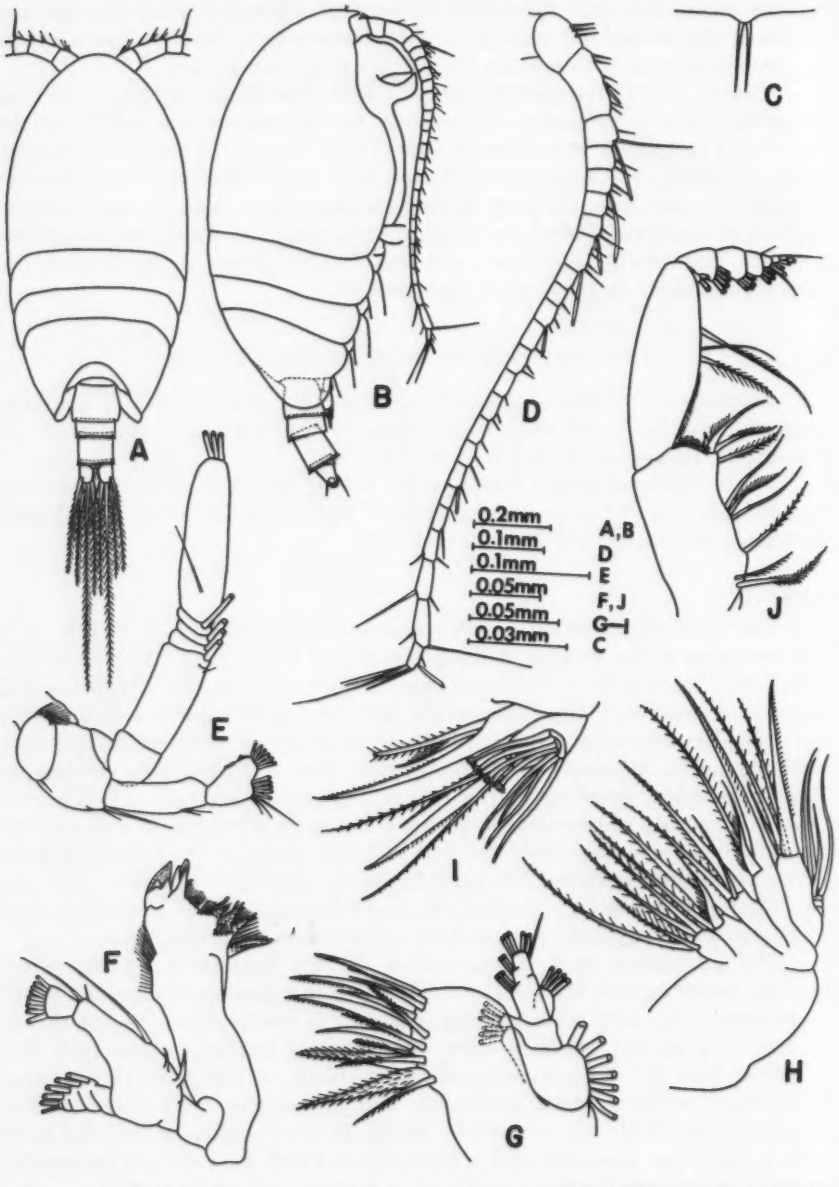

FIG. 4. *Tharybis fultoni*, new species. Female: A, habitus, dorsal view; B, habitus, lateral view; C, rostrum; D, antennule; E, antenna; F, mandible; G, maxillule; H, maxilla, external aspect; I, distal portion of maxilla, internal aspect; J, maxilliped.

The 2nd and 3rd inner lobes are similar; each has 3 setae. The external lobe and the exopod are relatively small and bear 8 and 4 setae, respectively. The maxilla (Fig. 4H, I) has 5 prominent lobes; the middle 3 carry 3 spines each and the others 4 spines each. The endopod has 8 sensoriform filaments; 4 of these are vermiform and longer than the remaining 4, which have a specialized tip. The maxilliped (Fig. 4J) consists of a large basipod and a small endopod. The basis is smoothly swollen, with the greatest thickness at mid length.

The 1st leg (Fig. 5A) has a 1-segmented endopod and a 3-segmented exopod. Each of the exopodal segments has a spine that is about as long as the combined lengths of any 2 exopodal segments. The 1st exopodal segment lacks internal setae. The 2nd leg (Fig. 5B) has a 2-segmented endopod and a 3-segmented exopod. On the posterior surface of the leg are found 3 rows of spinules on the 2nd endopodal segment and a row of spinules on the 3rd exopodal segment. The 3rd (Fig. 5C) and 4th (Fig. 5D) legs are similar in that both the endopod and the exopod are 3-segmented, but they are different in the ornamentation with spinules. That is, in the 3rd leg the 2 distal segments of the endopod and the 3rd segment of the exopod have on their posterior surface a row of spinules each, whereas these segments of the 4th leg have 2 or 3 patches of spinules.

The 5th pair of legs (Fig. 5E) is symmetrical; each leg has 3 segments. The distal segment is slightly more than twice as long as the combined lengths of the proximal 2 segments and is forked distally into 2 strong, blunt spines. The segment bears an internal spine which is located distally and almost equal in size to the adjacent terminal spine, and it also bears a small triangular spine halfway along the external margin.

Male

The specimens measure 1.09 to 1.12 mm long, and the ratio of the lengths of the prosome and the urosome is about 69: 31. The body (Fig. 5F, G) is similar to that of the female, but the postero-lateral processes of the metasome are inconspicuous, the urosome consists of 5 instead of 4 free segments, and the caudal rami are longer.

The antennules reach as far as the end of the metasome. In the right antennules (Fig. 5G) segments 20 and 21 are fused, whereas these segments are separated in the left antennules. The antenna and the mouth parts are similar in anatomical details to those of the females. The 1st to 4th legs are also similar to those of the female except that in the 4th leg (Fig. 5H) the coxal seta is very small, the 2nd endopodal segment is relatively long, and the endopod has no spinules on the posterior surface.

The 5th pair of legs (Fig. 5I, J) is asymmetrical; the right leg is biramose and the left uniramose. In the right leg the endopod consists of a long, single segment of a sickle shape, and the exopod of 2 segments; of these the distal one is small but highly complicated in structure. The left leg is about as long as the exopod of the right and is 4-segmented. The proximal segment is fused with the coxa of the right leg, and the distal segment is ear-shaped. The 3rd segment bears a falciform process in addition to a triangular process.

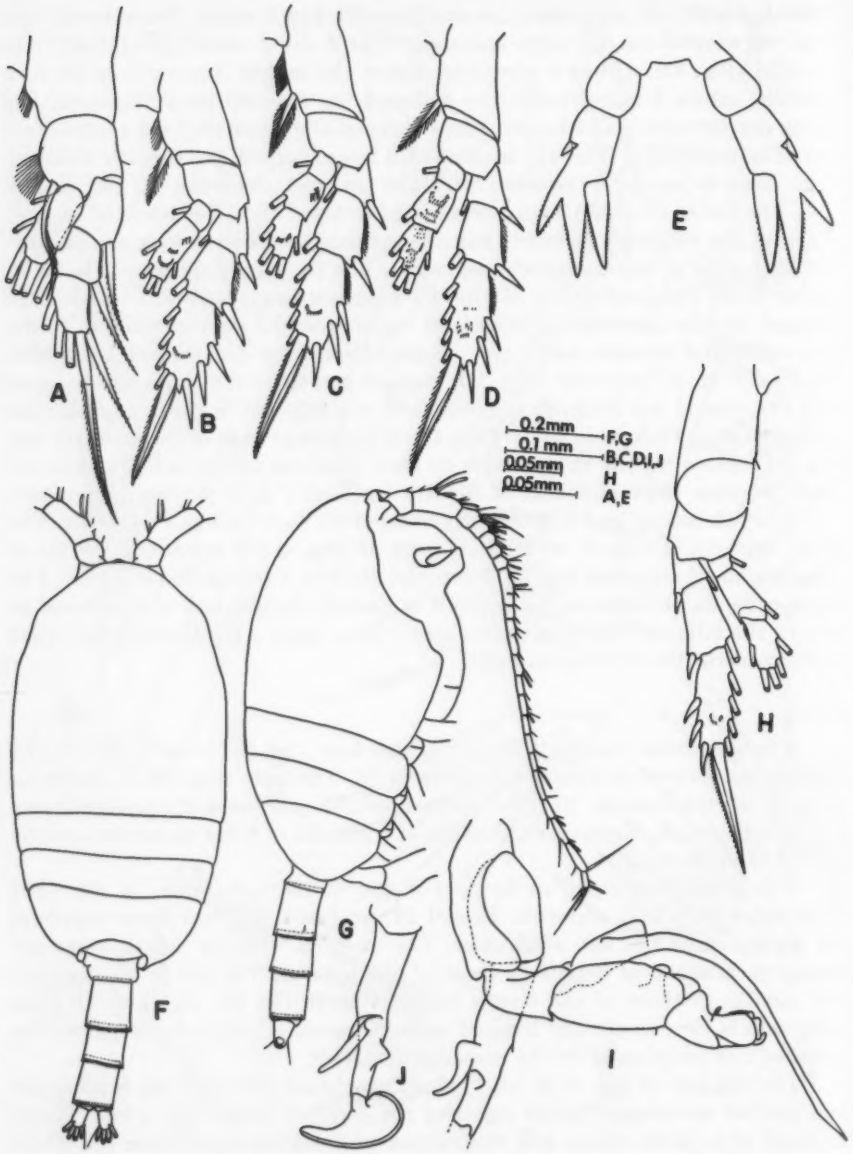

FIG. 5. *Tharybis fultoni*, new species. Female: A, 1st leg; B, 2nd leg; C, 3rd leg; D, 4th leg; E, 5th pair of legs. Male: F, habitus, dorsal view; G, habitus, lateral view; H, 4th leg; I, 5th pair of legs, anterior aspect (distal segment of left leg is omitted); J, distal portion of left 5th leg.

REMARKS

Tharybis fultoni is the 3rd species of the genus, the 2 previously described being *T. macrophthalma* Sars, 1902, from Norwegian waters and *T. sagamiensis* Tanaka, 1960, established on a single female from Japanese waters. The female of *T. fultoni* is easily distinguished from that of the others by the 5th pair of legs, which is characterized by the exceptionally large spines. The male may be distinguished from that of *T. macropthalma*, the only male previoulsy known in the genus, by the presence of a falciform process on the 3rd segment of the left 5th leg among other characters.

The family Tharybidae, of which *Tharybis* is the type genus, has been reviewed by Fleminger (1957), who provided a synoptic key to the genera.

Tharybis fultoni has been named in honor of Mr. John Fulton.

ACKNOWLEDGMENTS

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The Sodium, Potassium, and Water Content of the Flesh of Sockeye Salmon (*Oncorhynchus nerka*) in Relation to Sexual Development and Starvation[1,2]

By N. Tomlinson, J. R. McBride, and S. E. Geiger

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver, B.C.

ABSTRACT

The influence of feeding on the changes that occur in the skeletal muscle content of sodium, potassium, and water in sockeye salmon (*Oncorhynchus nerka*) during sexual development and spawning has been investigated. Feeding has little, if any, effect on the changes. Thus it appears that the period of starvation these fish undergo during their spawning migration under natural conditions is not the major cause of the degeneration of their skeletal muscle, but rather that these changes are associated in some manner with the development of the gonads, presumably through the mediation of hormones.

INTRODUCTION

In the course of their spawning migration in fresh water, during which time they do not feed, the flesh sodium concentration and water content of sockeye salmon increase, and the flesh potassium concentration decreases (MacLeod et al., 1958; Idler and Bitners, 1959). MacLeod et al. (1958) suggested that the changes in sodium and potassium concentrations might be under the control of hormones associated with sexual development; but similar changes, including those in flesh water content, have been observed in small, starved Atlantic cod that were apparently not undergoing sexual development (Love, 1958, 1960; Reay, 1963). The changes were, however, much more marked in larger, starved cod in which sexual development was proceeding. An increase in the water content of the flesh of American plaice that is apparently related to spawning has been described by Templeman and Andrews (1956). In mammals, edema and accumulation of salt are associated with starvation (man) (Keys et al., 1950); increased total muscle sodium and water, and decreased potassium are associated with aging (rats) — effects which can be induced in young neurohypophysectomized, adrenalectomized animals by administration of aldosterone (Friedman, et al., 1966); and cyclical increases in retention of salt and water are associated with the menstrual cycle (man) — effects which can be induced by injection of sex hormones (Thorn et al., 1958).

Although it appears to be generally accepted that Pacific salmon do not feed after entering fresh water on their spawning migration, it has been found that sockeye salmon do so when they are held in captivity in fresh water and are offered suitable food (McBride et al., 1963, 1965). Under such conditions the majority of the fish of both sexes soon begin to feed, many of them

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voraciously. This finding has made it possible to investigate the influence of feeding on various changes that occur in the fish during the course of the sexual development associated with spawning (McBride et al., 1965; McBride, 1966). Although feeding was found to be distinctly beneficial to several organs, extensive degeneration of the skeletal muscle occurred in both fed and unfed fish (McBride et al., 1965). However, in view of the known nutritional requirements of salmon (Halver, 1963), it appeared possible that the food might have been nutritionally inadequate, although it consisted of marine zooplankton (predominantly *Euphausia pacifica*) which at times form a large part of the natural food of sockeye salmon at sea (Allen and Aron, 1958). In view of this possibility, the work has been continued, but utilizing food that could be expected to be adequate. The results of that portion of the investigation dealing with the water, sodium, and potassium content of the flesh are presented below.

MATERIALS AND METHODS

Migrating, adult sockeye salmon were taken from the Great Central Lake run on Vancouver Island, B.C. Methods used in their capture, transportation, and maintenance in captivity have been described elsewhere (McBride et al., 1963, 1965; McBride, 1966). Rainbow trout (*Salmo gairdneri*) were obtained from a commercial trout farm.

In the 1964–65 season, salmon were fed a defined diet (Woodall and La Roche, 1964). In 1965–66 they were fed a mixture of canned salmon, beef liver, beef heart, Pablum, and water in a ratio of 8:4:4:1:1 (referred to below as the mixed meat diet). Each diet was initially supplemented with 20% (by weight) of marine zooplankton. The supplement was reduced as the fish began feeding, and was eliminated entirely after a few days (McBride, 1966). The salmon were fed to repletion once or twice daily. By means of a count of portions of the food taken by individual fish it was estimated that 10% of them consumed food to the extent of 1–2% of their body weight daily, and 80% of them, 0.5–1%. The remainder took very little food or none at all. Trout were fed 2% of their initial body weight per day. In consequence of their growth, trout were thus being fed less than 1% of their body weight per day by the time the work with them was terminated.

The defined diet contained 1% sodium chloride; the mixed meat diet, 1.5%; and the frozen marine zooplankton, 2.4%. The aquarium water supply contained about 3 μg sodium/ml.

The water content of flesh was determined by drying 10-g samples for 4 days at 105 C. Sodium and potassium were measured by flame spectrophotometry.

RESULTS

Data obtained during the season 1964–65, when the defined diet was fed, are presented in Table I. Results in 1965–66, when the meat mixture was fed,

TABLE I. Influence of feeding on the water, sodium, and potassium content of the dorsal muscle of spawning sockeye salmon.[a]

Condition of fish	Date	No. of fish	Water (%)	Sodium (*mg/g muscle*)	Potassium (*mg/g muscle*)
	1964				
Unfed, taken from river in early migration	July	8	73.6	0.24	5.00
Unfed, unspawned	Oct.–Nov.	5	82.1	0.64	4.27
Fed, unspawned	Oct.–Nov.	6	82.0	0.56	4.40
Unfed, spawned	Oct.–Nov.	6	83.5	0.73	4.20
Fed, spawned	Oct.–Nov.	12	81.9	0.64	4.28
Fed, spawned	Dec.	3	83.7	1.38	4.33
	1965				
Fed, spawned	Jan.	3	88.5	1.55	4.43
	1964				
Unfed, spawned under natural conditions in Great Central Lake	Oct.	5	81.0	0.61	3.63

[a]Fish fed the defined diet.

were practically identical. Clearly, feeding exerted very little effect on the course of events in the flesh during sexual development and spawning. The water and sodium content increased and the potassium content decreased in the flesh of fed and unfed fish to about the same extent. However, as was observed in the earlier work in which only marine zooplankton were fed (McBride et al., 1965), fed fish survived for a longer period after spawning than did unfed fish. Consequently it was possible to obtain data for the former over a longer period of time. It was found that the water and sodium content of the flesh of these fish continued to increase during the extended observation period, but no further reduction in potassium content was detected.

Data for water and sodium in flesh of fish spawned in Great Central Lake under natural conditions were similar to those of fish spawned in the laboratory at about the same time, but their flesh potassium content was lower. Whether this is a real difference, perhaps reflecting a greater degree of activity by fish in the wild, or simply fortuitous as a result of the small number of naturally spawned fish examined, is not known. It was not possible to re-examine this particular aspect in 1965–66. However, the potassium content of the flesh of fish held in captivity can eventually fall even lower, and the water and sodium contents can reach remarkably high values, e.g. in four unfed, unspawned fish killed in February 1964, the potassium, sodium, and water contents of the flesh were 2.9 $\pm$ 0.55 mg/g flesh, 1.8 $\pm$ 0.3 mg/g flesh, and 92.5 $\pm$ 1.4%, respectively.

During the course of the work in 1965–66 an examination was made of the ability of the two diets to support the growth of rainbow trout (Table II). In a period of 6 months, trout grew rapidly on either diet, whereas all but

TABLE II. Ability of two diets to support growth of rainbow trout.[a]

Treatment	Weight[b]		Final water content of flesh
	Initial	Final	
Fed:[c]	(g)	(g)	(%)
Mixed meat diet	93.5 (68–113)	274 (95–369)	77.9
Defined diet	95.0 (59–124)	232 (149–333)	77.9
Unfed	93.0 (63–142)	57 (41–73)	86.6

[a]Each treatment group contained 30 fish at the beginning of the experiment on June 24, 1965. Twenty-six of those fed the mixed meat diet and 28 of those fed the defined diet survived until the experiment was terminated, December 29, 1965. Only 2 in the unfed group survived, the other 28 dying between August 16 and October 15, 1965.

[b]Average initial and final weights, with the range in brackets.

[c]For composition of the diets see Methods and Materials. Fish were supplied with food at the rate of 2% of their average initial weight daily.

two fish in an unfed control group died. The surviving unfed fish had lost weight and their flesh water content was high, whereas the fed fish had gained weight (by 193% on the meat diet and 144% on the defined diet) and their flesh water content was normal.

DISCUSSION

Although the findings described above can hardly be regarded as conclusive evidence that starvation plays no part in bringing about the degenerative changes observed in the skeletal muscle of migrating, spawning sockeye salmon, it seems reasonable to expect, particularly in view of the experimental results with rainbow trout, that the muscle of the feeding sockeye should have shown much greater benefit than it did from the food taken if starvation was the major influence responsible for the changes.

The evidence available indicates that the development of the gonads is in some manner directly concerned in the changes, for the skeletal muscle of sexually developing sockeye has previously been found to show marked benefit from feeding after the fish were gonadectomised, and a similar effect has been observed in unspawned male sockeye which commenced feeding some 6 months after the time when they would normally have spawned (McBride et al., 1963). Presumably, as MacLeod et al. (1958) have suggested, the changes in the flesh are mediated by hormones, but little information regarding the part played by the endocrines of fishes in this respect is available. Adrenal corticoids injected into trout have been shown to influence the concentrations of sodium and potassium in the blood and the excretion of sodium through the gills (Saulding, *cited by* Chester Jones, 1956; Chester

Jones et al., 1959), but the relationship of these findings to the normal physiology of salmon remains uncertain. Idler et al. (1959) found that none of the more probable electrolyte-regulating hormones was present in significant quantity in the sockeye salmon plasma they examined, and these investigators suggested the possibility that cortisone and cortisol, which were present, might regulate mineral metabolism in the fish, at least during their spawning migration. On the other hand, Phillips et al. (1959) reported the occurrence of the potent electolyte-regulating hormone, aldosterone, in salmon plasma. The sex hormones have been shown to exert a "sodium-retaining" effect in dogs (Thorn et al., 1938) and certain of these hormones have been identified in the plasma (Schmidt and Idler, 1962) and in the ovaries (Botticelli and Hisaw, 1964) of sockeye salmon, but their electrolyte-regulating role in fish does not appear to have been investigated, although Idler et al. (1961) have reported an increase in the water content of the flesh of sockeye injected with 11-ketotestosterone. Finally, there is some evidence that thyroid hormone is concerned in the control of salt and water in teleosts (Hoar, 1957).

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A Generalized Computer Program For the Ricker Model of Equilibrium Yield Per Recruitment[1,2]

By G. J. Paulik and William H. Bayliff

College of Fisheries, University of Washington, Seattle, Washington,
and
Inter-American Tropical Tuna Commission
Scripps Institution of Oceanography, La Jolla, California

ABSTRACT

The Ricker method for predicting the yield per recruitment from a stock of fish under various conditions is superior to that of Beverton and Holt (1957) under most conditions because it permits greater flexibility of the input of growth and mortality data. Such useful devices as the isopleth diagram and the eumetric fishing curve, usually associated with the Beverton and Holt method, can be used also with the Ricker method. A computer program for the Ricker method is described, and its use is demonstrated with an example.

INTRODUCTION

One of the most useful concepts that fisheries science has produced is that of equilibrium yield per recruitment. This concept has proven itself not only as a highly stimulating theory about the productivity of exploited fish stocks but also as a basic working tool for fisheries managers. The manager is able to use this concept to further his understanding of the fishery and to make predictions as to how the yield per recruitment will respond to changes in the type of fishing gear and/or the intensity of fishing.

Ricker (1958, p. 205–228) provides an excellent history of the equilibrium yield per recruitment concept. Baranov (1918) (*cited by* Ricker, 1958) was probably the first to formulate the dynamics of exploited fish populations with precisely defined mathematical symbols, and significant advances were made by Thompson and Bell (1934) and Ricker (1945). Beverton and Holt (1957) and Ricker (1958) are chiefly responsible for the further development of this concept to the present.

To gain a better perspective of the present position of the equilibrium yield theory and to make clear the reason for the present paper, it is advisable to examine the derivation of the basic model. The rate of yield of a stock at time t is expressed by

$$dY_W = F_t N_t w_t dt \qquad \ldots\ldots(1)$$

where Y_W = yield in weight, F_t = instantaneous fishing mortality rate, N_t = number of fish present, and w_t = average weight of the individual fish.

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By integrating over the fishable life span of a group of recruits entering the fishery, the total yield in weight produced by these recruits can be computed. If the annual recruitment is constant and the other conditions are stable for a period equal to the fishable life span, this yield will also be equal to the total yield during 1 year from all year-classes. The stability assumptions imply that the annual yields per recruitment do not change from year to year, and hence the name "equilibrium yield per recruitment."

Beverton and Holt (1957, p. 308–330) present a compact and elegant mathematical solution for the equilibrium yield per recruitment, expressing the three factors on the right side of equation 1 as simple functions of time. By choosing suitable functions, the resulting equation can be integrated to obtain a closed algebraic expression for the total equilibrium yield per recruitment. The primary value of such an expression is that it can be easily manipulated to show how the equilibrium yield per recruitment responds to changes in parameters representing fishing intensity, age of entry into the fishery, growth rate, etc. They introduced some highly useful methods for expressing their results, such as isopleth diagrams and the eumetric fishing curve. Beverton and Holt's (1957) method sacrifices a great deal of flexibility to achieve neatness and elegance. This is unfortunate, for the available data make it clear that the growth and mortality of a species of fish usually cannot be represented by simple formulas except as rather crude first approximations.

The strength of Ricker's (1958, p. 208–217) method is its flexibility, achieved at the sacrifice of mathematical elegance, while its practical disadvantage is the massive amount of tedious arithmetic which it requires. The sheer bulk of calculations necessary to construct an isopleth diagram based on Ricker's method has been too great to allow its widespread use. Recent developments in computers, however, allow a reexamination of the relative advantages and disadvantages of the Beverton and Holt (1957) and the Ricker solutions to the problem of equilibrium yield per recruitment, for modern digital computers now make it relatively simple to use Ricker's method.

The purpose of this paper is to describe the implemantation of Ricker's method on a digital computer, and to point out the existence of a computer program which will carry out all the necessary calculations. The program described has been used in graduate classes in population dynamics at the College of Fisheries, University of Washington, since 1964.

DERIVATION OF THE GENERALIZED RICKER MODEL

The total yield in weight can be calculated by integration of equation 1

$$Y_W = \int_{t_0}^{t_n} F_t N_t w_t \, dt \qquad \ldots\ldots(2)$$

where $t_n - t_0$ = maximum time an individual of the species remains in the fishery. Ricker (1958) approximates the solution arithmetically. First he divides the

fishable life span into a number of segments "such that neither growth rate nor mortality rate are changing too rapidly within any period." These segments do not need to be of the same length. The weight that the fishery removes from the stock during a segment of length $\Delta t_i = t_i - t_{i-1}$ is

$$\Delta Y_i = \overline{F}_i \overline{N}_i \overline{w}_i \Delta t_i \qquad \ldots\ldots(3)$$

where $\overline{F}_i$ is defined as the average of the time-specific instantaneous fishing mortality rates at the beginning and the end of the segment. $\overline{N}_i$ and $\overline{w}_i$ are defined similarly, i.e.

$$\overline{F}_i = \frac{F_{t_{i-1}} + F_{t_i}}{2} \qquad \ldots\ldots(4a)$$

$$\overline{N}_i = \frac{N_{t_{i-1}} + N_{t_i}}{2} \qquad \ldots\ldots(4b)$$

$$\overline{w}_i = \frac{w_{t_{i-1}} + w_{t_i}}{2} \qquad \ldots\ldots(4c)$$

(In Ricker's notation the product $\overline{N}_i \overline{w}_i$ is written as $\overline{W}_T$ and the product $\overline{F}_i \Delta t_i$ as p_T.) The total equilibrium yield per recruitment is obtained by summing the catches in each segment over the total number of segments, n, in the fishable life span

$$Y_W = \sum_{i=1}^{n} \Delta Y_i \qquad \ldots\ldots(5)$$

For functions that cannot be integrated by ordinary methods, this type of numerical integration employing a digital computer is a quick, simple, and reliable method for obtaining the intrgral, the degree of accuracy depending on the lengths of the segments. Its primary virtue is that it eliminates concern about the forms of mathematical functions to describe the variations in F, N, and w with time.

There is an alternative form of the yield per recruitment equation which is useful when the stock is "increasing or decreasing strictly exponentially," as noted by Ricker (1958, p. 209, footnote 2). In this case the functions in equation 1 would be expressed by

$$F_t = F_i \qquad \ldots\ldots(6)$$

$$N_t = N_{t_{i-1}} e^{-(F_i + M_i)(t - t_{i-1})} \qquad \ldots\ldots(7)$$

where M_i = instantaneous natural mortality rate, and

$$w_t = w_{t_{i-1}} e^{g_i (t - t_{i-1})} \quad \ldots\ldots(8)$$

where g_i = instantaneous growth rate for $t_{i-1} \leqslant t \leqslant t_i$. M_i and g_i are both assumed to be constant during the ith segment of the fish's life. Integration of the formula between t_{i-1} and t_i results in the following equation for the yield per recruitment during the ith segment:

$$\Delta Y_i' = F_i N_{t_{i-1}} w_{t_{i-1}} \left[\frac{e^{(g_i - F_i - M_i)\Delta_i} - 1}{g_i - F_i - M_i} \right] \quad \ldots\ldots(9).$$

Ricker (1958, p. 209. footnote 2) points out that when the rate of growth decreases through most of the life of the fish, as is usually the case, this method is inferior to the one described by equation 3.

ΔY_i (equation 3) will henceforth be designated as the arithmetic mode yield and $\Delta Y_i'$ (equation 9) as the exponential mode yield. If the product $(g_i - F_i - M_i)\Delta_i$ is small, the arithmetic mode and the exponential mode computations give practically the same result, as they both approach the exact solution, which could be obtained only by integration. (This result, incidentally, agrees with Ricker's advice that the segments should be small if the rates are changing rapidly.) The more general arithmetic yield formula may be conveniently thought of as a method of approximating any type of mortality and growth functions for which the accuracy will become progressively greater as the Δ_i become smaller.

In the general computer program which has been prepared to carry out these computations, the user can control both the mode of computation and the size of the individual segments for each of the various rates involved. Since F_i, M_i, and g_i can take on any values in the intervals, the procedure makes it possible to express directly the total yield from a given number of recruits for any complex and variable patterns of fishing mortality rates, natural mortality rates, and growth rates which occur during the life span of the fish.

ISOPLETH DIAGRAMS FOR YIELD PER RECRUITMENT

Beverton and Holt (1957, Figs. 17.14 and 17.26) made yield per recruitment isopleth diagrams, placing the instantaneous fishing mortality rate, F, on the abscissa and the age at entry, $t_c (= t\rho')$, on the ordinate, and computing values of the equilibrium yield per recruitment for each pair of F and t_c values. Contour lines were drawn between equal values of yield per recruitment. This response surface is a means of visualizing the yield per recruitment as a function of these two variables, and enables the fishery manager to predict the manner in which the yield per recruitment will respond to changes in the fishing effort and age at entry.

The age at entry is easily incorporated into equation 1 by setting $F_i = 0$ for any time segment, Δ_i, for which the age at the end of the segment is less than or equal to t_c.

The pattern of fishing mortality is usually more complex. F is the product of the fishing effort, f, and the coefficient of catchability, q. If both of these are constant after the age of entry into the fishery, the method of Beverton and Holt is easily adopted. This is accomplished by taking some arbitrarily chosen value of F, such as its present value, as unity, and then computing the yield per recruitment at various multiples of F which are of interest. In this way it can be estimated what the yield per recruitment would be if the fishing effort were halved, doubled, etc. It is likely, however, that f, q, or both of these will vary after entry into the fishery, so that more complex patterns of F will result. In the temperate zones, at least, the fishing effort for most species is seasonal; in many cases there is no fishing during part of the year, so f would be zero at these times. Thus there can be introduced an array of values of f corresponding to the effort during different time segments of an arbitrarily chosen base year. The availability and vulnerability, which determine the coefficient of catchcbility, may vary according to the time of the year and the age of the fish. The value of q before entry into the fishery would be zero, of course. The vulnerability of a species caught in trawls or gill nets is likely to increase gradually from zero over a period of several weeks, months, or years as the fish are recruited into the fishery. Some species become more or less available or vulnerable each year near the time of spawning, and individuals of some species become less available or vulnerable when they reach larger than average size. Thus there can be introduced an array of values of q for the life span of the fish, and an array of values of F for its life span, corresponding to the products of the respective values of f and q. This vector can be multiplied by various fractions and whole numbers to represent changes in the fishing effort relative to the base year. Using this system the yield per recruitment isopleth diagram can be plotted as a function of the age at entry into the fishery and a multiplier which is applied to the vector of instantaneous fishing mortality rates that occur during the life span of the fish.

Yield isopleth values for large multipliers should always be carefully checked and interpreted with caution. Assuming that a multiplier of 1 represents the present level of fishing, use of a multiplier of 10 or 20 implies that it is possible to forecast the change in equilibrium yield that would occur if fishing effort were increased 10- or 20-fold. If experience and data are limited to levels of fishing not greatly different from the present level, any forecast based on an extrapolation of this magnitude must be viewed with suspicion. Basic biological assumptions underlying the equilibrium yield concept may be invalidated by a massive increase in the fishing mortality rate. Recruitment may be affected or natural mortality rate or growth rate may change as a result of the change in population size caused by an increase in the rate of fishing. If an exploited species is reduced to a low level of abundance, its relationships with other species sharing the same environment may change

drastically. Large multipliers may also affect computational accuracy. The arithmetic mode of computation (equation 3) may grossly overestimate the actual equilibrium yield unless the Δ_i segments are adjusted so that the products $(g_i - F_i - M_i)\Delta_i$ are small. When an extremely large multiplier is used, for all practical purposes the population is decreasing exponentially and the exponential mode of computation given in equation 9 is appropriate. As a general rule, when there is some doubt concerning the accuracy of the results, it is advisable to compute yields using both the arithmetic and exponential modes of computation. If the yields so obtained differ and the reason for the difference is not readily apparent, the Δ_i segments should be shortened.

COMPUTER PROGRAM

The input for the program consists of: (1) an arbitrarily chosen initial weight of recruits; (2) growth, natural mortality, and fishing mortality data for chosen time intervals; (3) trial values of age at entry; and (4) trial "multipliers".

The growth data may be in the form of either instantaneous growth rates or weights at chosen time intervals. In the latter case the computer will calculate instantaneous average growth rates during the interval by

$$g_i = ln\left(\frac{w_{t_{i-1}}}{w_{t_i}}\right) \Big/ (t_i - t_{i-1}) \qquad \ldots\ldots(10)$$

where g_i = average instantaneous growth rate for interval i, and $w_{t_{i-1}}$ and w_{t_i} = average weights of the individual fish at the beginning and end of interval i, respectively. Similarly, the natural mortality data may be in the form of either instantaneous mortality rates or numbers of fish surviving at chosen time intervals. In the latter case the computer will calculate the instantaneous average natural mortality rates during the period by

$$M_i = -ln\left(\frac{N_{t_{i-1}}}{N_{t_i}}\right) \Big/ (t_i - t_{i-1}) \qquad \ldots\ldots(11)$$

where the notation is equivalent to that of equation 10. The fishing mortality data must be entered as instantaneous rates. The intervals employed for any rate need not be of equal lengths, nor need the intervals used for the different rates bear any particular relationship to one another. The maximum number of intervals which may be employed are as follows: growth, 99; natural mortality, 99; fishing mortality, 400.

As many as 20 trial values for the age at entry can be used.

The multipliers are scalars for the vector of instantaneous fishing mortality rates described above. As many as 20 trial multipliers can be employed.

The output consists of a matrix of equilibrium yield per recruitment values for each combination of multiplier and age at entry. These values can

be calculated by either the arithmetic mode (equation 3) or the exponential mode (equation 9), as specified by the user. Because of the restrictions the matrix will contain a maximum of 400 values. If necessary, however, more values can be obtained by repeating the problem with different multipliers and/or different values for the age at entry.

The output also includes the biomass of the stock at specified times when there is no fishing mortality. This biomass vector is useful for determining the optimum harvest time for such organisms as oysters living in the intertidal zone, where it is possible to harvest all the animals larger that a certain size at one time (i.e. the instantaneous fishing mortality rate can be infinite).

The use of the computer program is illustrated by applying it to Ricker's (1958, p. 210–217) yield per recruitment example for bluegills in Muskullunge Lake, Indiana.

To reproduce Ricker's entering stock size of 1000 g the initial number of recruits is entered as 1000 divided by 13 g (the initial weight), or 76.9 fish. All the results are given in terms of an entering biomass of 1000 g of recruits.

The pattern of growth is obtained from Ricker's Table 10.3 The data introduced into the computer are pairs of values:

Age in years	2.000	2.125	2.250	....	6.000
Weight in grams	13	29	44	....	158

From these numbers the computer calculates the instantaneous growth rates, which are used for the calculations and printed in the output:

Growth interval	1	2	3	....	20
From	2.000	2.125	2.250	....	5.500
to	2.125	2.250	2.375	....	6.000
Instantaneous growth rate (annual)	6.4188	3.3352	2.2100	....	0.0000

In this program it is necessary to express all the data with the same time scale, which in this case is years. Thus the product of the interval length and the instantaneous annual growth rate gives Ricker's g for the corresponding interval except slight discrepancies due to rounding errors (e.g. Ricker gives $g_1 = 0.81$ while the product $(0.125)(6.4188)$ gives $g_1 = 0.80235$, the correct value to five decimal places).

The instantaneous natural mortality rates are taken from Ricker's Table 10.3:

Age in years at start of interval	2.000	5.125	5.250	5.375	5.500	6.000
Instantaneous natural mortality rate (annual)	0.600	0.840	1.120	1.600	2.400	–

No natural mortality rate is given for the last age value, as this defines the upper limit of the last interval. Since the natural mortality rate is constant from 2 to $5\frac{1}{8}$ years of age it is necessary to specify only one natural mortality interval for this period. The sum of Ricker's q (=M) values between 2 and $5\frac{1}{8}$ years is equal to the product (0.600) (5.125 – 2.000).

The instantaneous fishing mortality data are handled in exactly the same way as the instantaneous natural mortality data:

Age in years at start of interval	2.000	2.125	2.250	....	6.000
Instantaneous fishing mortality rate (annual)	0.00	0.32	1.12	....	–

Once again it will be noted that the sum of Ricker's p (=F) values for any given interval is equal to the product of the corresponding annual rate and the length of the interval.

The remainder of the input consists of values of the multipliers and of the age at entry, For this example multiplier values of 0.25, 0.50, 0.75, 1.00, 1.50, ... 5.00, 6.00, ..., 10.00, and age-at-entry values of 2.125, 2.250, 3.125, 3.250, 4.125, 4.250, 5.125, and 5.250 were selected. The combination of 17 multipliers and 8 ages at entry produces a yield per recruitment matrix of 136 values (Fig. 1). The value of 2273.45 g for a multiplier of 1.00 and an age at entry of 2.125 years corresponds to the yield per recruitment of 2287 g given by Ricker. The discrepency is due to errors caused by Ricker's use of 2-place natural logarithms in his growth rate computations. When the instantaneous growth rates given in his Table 10.3 were used as input to the program a value of 2287.66 g was obtained, which is in almost exact agreement with Ricker's value.

The eccentric shape of the isopleths, as compared to those of Beverton and Holt (1957), is chiefly the result of the seasonal nature of the fishery. The fish of catchable size are all assumed to be 2.125, 3.125, 4.125, or 5.125 years old on June 16, when the season opens, and 2.375, 3.375, 4.375, or 5.375 on September 15, when it closes. Thus if the age at entry is set at $2.375 \leqslant t_c < 3.125$ the yield per recruitment will be the same as when the age at entry

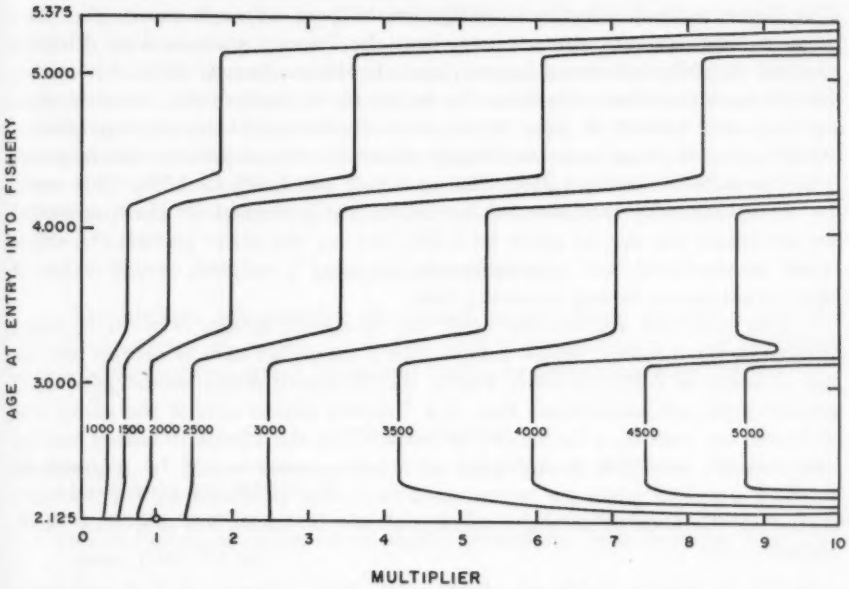

FIG. 1. Yield per recruitment isopleth diagram showing the yield of bluegills per 1000 g of recruits theoretically obtainable from a 3-month fishing season, for any intensity of fishing up to 10 times that in effect in Ricker's Table 10.3 and any age at entry from 2.125 to 5.375. The small circle on the abscissa represents the status of the fishery as shown in Ricker's Table 10.3.

is set at $t_c = 3.125$ because there is no fishing between September 16 and June 15. For this reason the isopleths are vertical at $2.375 \leqslant t_c \leqslant 3.125$, $3.375 \leqslant t_c \leqslant 4.125$, and $4.375 \leqslant t_c \leqslant 5.125$.

A copy of the program and a detailed explanation of its use can be obtained from Mrs. Helen Bailey, Fisheries Research Institute, University of Washington, Seattle, Washington 98105.

DISCUSSION AND CONCLUSION

The Ricker method is superior to that of Beverton and Holt (1957) in that it permits realistic simulation of growth and mortality when these cannot be expressed as simple functions of time. For instance, Bayliff (1966) has used this method for calculation of the yield per recruitment of the anchoveta in the Gulf of Panama. For this species the life span is short, the growth is seasonal, and the instantaneous rate of fishing mortality varies considerably over the course of the fishing season. It is apparent that for this species and many others the yield per recruitment under different conditions could best be predicted with Ricker's method.

The Ricker method permits more flexibility in investigating the effects of different management policies than does the method of Beverton and Holt.

The latter method permits investigation only of adjustments of the total fishing effort and the age at entry into the fishery, whereas with Ricker's method the effect of closed seasons can also be evaluated. With this model, for instance, the effect of opening the season for bluegills in the above example on August 1 instead of June 16 has been predicted. This was accomplished by changing the instantaneous fishing mortality rate to 0.00 for the intervals 2.125 to 2.250, 3.125 to 3.250, 4.125 to 4.250, and 5.125 to 5.250. This could be accomplished by the Beverton and Holt (1957) method for the first period by increasing the age at entry to 2.250, but for the other periods the effect could be predicted only approximately by using a reduced overall value of the instantaneous fishing mortality rate.

The yield per recruitment with the 1½-month season (Fig. 2) is lower than with the 3-month season except with a multiplier of 9 or greater and an age at entry of 2.250. In other words, the 1½-month season would produce a greater yield per recruitment than the 3-month season only if the effort was increased for both by a factor of 9 or more. With the 1½-month season and an age at entry of 2.250, a multiplier of 2.5 or greater would be required to produce a greater yield per recruitment than that produced by the 3-month season at the level of fishing intensity shown in Ricker's Table 10.3 (multiplier = 1).

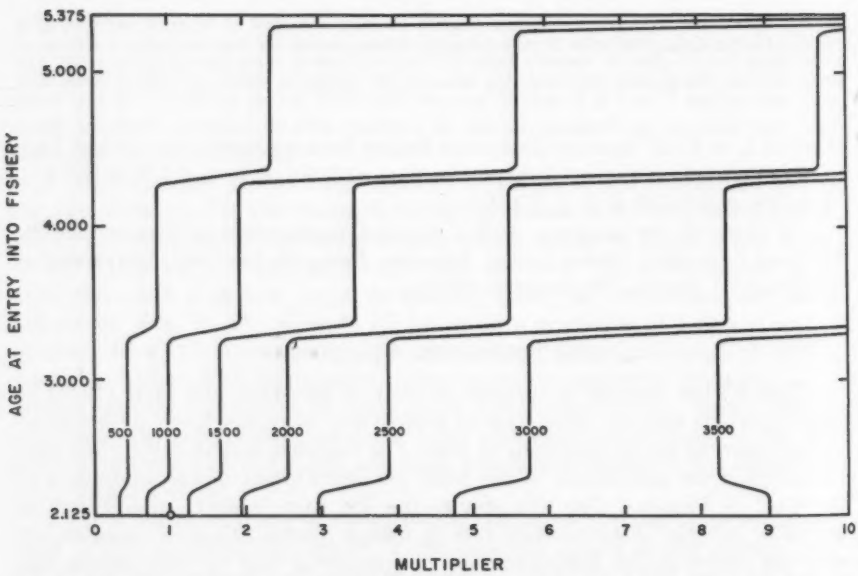

FIG. 2. Yield per recruitment isopleth diagram showing the yield of bluegills per 1000 g of recruits theoretically obtainable from a 1½-month fishing season, for any intensity of fishing up to 10 times that in effect in Ricker's Table 10.3 and any age at entry from 2.125 to 5.375. The small circle on the abscissa represents the status of the fishery as shown in Ricker's Table 10.3.

In general, Beverton and Holt's (1957) method is probably nearly as suitable as Ricker's for such long-lived species as the plaice, where seasonal variations in the growth and/or mortality may be averaged out over the fishable live span, and where the period of entry into the fishery is short relative to the fishable life span. However, as knowledge of the biology of fishes increases and management policies become more complicated it is anticipated that the shortcomings of the Beverton and Holt method will become more apparent, and that the Ricker method will be used more frequently.

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Estimation of Marine-Borer Attack on Wooden Surfaces[1]

By C. C. Walden, I. V. F. Allen, and P. C. Trussell

Division of Applied Biology
British Columbia Research Council
Vancouver, B.C.

ABSTRACT

Bankia setacea prefer to attack upper horizontal wooden surfaces, whereas *Limnoria* spp. prefer the lower surfaces. Attack by *B. setacea* is heaviest at the mudline and decreases almost linearly with decreasing depth. Limnorial attack is heaviest in the intertidal zone, then is relatively constant with increasing depth. Statistical studies showed non-normal distribution of populations, with limited replicability, particularly for low *Bankia* counts. The desirable period of immersion is greater than 2 weeks, but in most commercial monitoring situations is limited to 1 month. Attack data on immersion specimens are compared with those on floating logs. On the basis of the results, procedures have been designed for monitoring attack in the commercial evaluation of impregnants, as well as for estimating population densities.

INTRODUCTION

Untreated wood immersed in the sea is subject to destruction by marine wood borers, most commonly by *Teredo* and *Bankia* spp., molluscs of the family Teredinidae, or by *Limnoria* spp., crustaceans of the family Limnoriidae. Species of the pholad genus *Martesia* and of the crustacean genus *Chelura* also find natural habitats in wood, but they are usually of minor significance, particularly in waters of the temperate zone. For protective measures to be effective, methods are required for detecting the presence of borers and for establishing their population densities. Because of difficulties associated with intertidal or underwater inspections, wooden test specimens are immersed in the sea and later withdrawn for microscopic examination.

Along the Pacific coast of North America, where Teredinidae and various species of *Limnoria* are of principal economic concern, quantitative measurements of marine-borer populations are required for research and protection programs. Where breeding data indicate fresh teredine attack, the logarithmic nature of teredine growth gives the logging operator 60 to 90 days in which to move logs to fresh water or otherwise dispose of them before economic damage ensues. In semi-permanent marine structures constructed of unimpregnated timbers and piles, sodium arsenite treatments (Allen et al., 1966) are scheduled on the basis of borer breeding cycles. Evaluation of marine impregnants necessitates that the test specimens be exposed to heavy borer attack. For some purposes, precise estimation of borer numbers is essential.

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For example, attack by the teredine borer *Bankia setacea* in excess of 100 per ft^2 will completely destroy timber or logs. As few as 1–10 young borers per ft^2 will destroy the valuable outer sappy layers of veneer logs and degrade them to lumber or pulp uses.

Although marine-borer attack normally is estimated numerically as the number of organisms attacking per square foot of exposed wood surface, statistical measures of the variation have not been given heretofore. The so-called Clapp panel is widely used, and consists of $1 \times 6 \times 12$-inch pine or Douglas fir boards immersed with the 6-inch dimension in the vertical plane. After immersion for 1–8 months, the teredine punctures and pholad or limnorial tunnels are counted microscopically. For the longer immersion periods, wood shrinkage may be rated in per cent. The origins of this panel, as listed in the annotated bibliography prepared by Clapp and Kenk (1963), are described in a number of mimeographed reports which are not generally available. Thus, the literature does not indicate the validity of this technique for gauging marine-borer attack.

This paper deals with various types of immersion specimens fo rmeasuring borer attack, and the uses and limitations of these specimens.

MATERIALS AND METHODS

Immersion Materials

The immersion materials used were blocks, plywood panels, wooden panels, and logs.

Blocks were Douglas fir cubes, sized to give an area on each face of 3.6 square inches, or a total area for the four flat-grained surfaces of 0.10 ft^2. Blocks were stapled through the end grain to tarred hemp lines (200-lb test) at 1-, 2-, or 5-ft intervals. When individual strings of blocks were immersed vertically, two of the planed surfaces of each block were in a vertical position, one in an upper horizontal position, and one down.

Plywood panels, 12 inches square, were cut from 3-ply Douglas fir plywood and drilled in the center of each panel face. These panels were mounted on iron frames so that when suspended in water, they were immersed in the horizontal plane at 1-, 2-, 3-, and 6-ft depths.

Wooden panels were $1 \times 6 \times 12$-inch straight-grained Douglas fir drilled through the center of each panel face, and mounted in groups of seven on a 5-ft length of $1\frac{1}{2} \times \frac{1}{4}$-inch steel channel protected against corrosion. Panels were bolted to the supporting iron channel, 2 inches apart, with the 12-inch length at right angles to the supporting bar.

Logs were western hemlock, approximately 12 inches in diameter by 10 ft in length.

Immersion Sites

Where feasible, studies involving *B. setacea* (the local teredine species) were undertaken in Vancouver Harbor during the fall and winter months,

the heaviest breeding season. Other immersion sites were Elliott Bay (Seattle Harbor) and Tacoma Harbor, where breeding of this borer is heavy throughout the year.

Studies involving *Limnoria* spp. were made in Alameda Harbor (San Francisco Bay), where limnorial breeding is heavy each spring and appreciable throughout the remainder of the year.

Immersion Procedures

Strings of blocks were weighted at one end and immersed vertically in the water. For strings with blocks at 5-ft intervals, the top block was positioned at mean lower low tide. Those strings with blocks at 1- and 2-ft intervals were suspended from floating structures, with the top block 1 ft below the water surface. The 12-inch-square plywood panels were immersed from a floating structure with the top panel in each frame 1 ft below the water surface and the bottom panel 6 ft below. Frames containing the $1 \times 6 \times 12$-inch panels, weighted at one end, were immersed at 20 ft below mean lower low tide. Logs were immersed in a floating position.

Except as otherwise noted, experimental materials were immersed for 1 calendar month, then removed and examined. Continuing data were obtained by immersion of fresh material. Individual floating logs, on the other hand were in sea water continuously from the data of immersion to date of removal, for periods from 4–9 months.

Laboratory Examination

After exposure to borer attack in the sea, smaller specimens were cleaned and dried. The planed surfaces of blocks and panels were subdivided by pencil into areas recognizable under the magnification of 10 to 100 diameters used with the stereoscopic microscope, and all the teredine punctures and limnorial tunnels on the planed surfaces were counted.

As closely as the ability of the experienced microscopist permitted, teredine borers were classified, in specific instances, on the basis of the depth of the *Bankia* pit and the extent of calcareous deposition on the pit walls, into the four following age-groups: 1 to 3, 4 to 5, 6 to 7, and 8 to 30 days.

After removal from the water, logs were stored in unheated storage until decay of *B. setacea* bodies was complete. A 2-ft long section was taken from each end of each log and discarded. The surface of another 2-ft section of each log was examined with a hand glass to determine the number of teredine punctures, using a wire probe to estimate the length of individual tunnels.

RESULTS

Settlement on Horizontal and Vertical Surfaces

Separate tabulation of teredine punctures on the upper and lower horizontal surfaces and the two vertical surfaces of blocks showed that establishment of young *B. setacea* occurs predominantly on the upper horizontal surfaces of wood (Table I). Immersions pertained to two locations in Tacoma

TABLE I. Numbers of *Bankia setacea* and percentages of totals attacking horizontal and vertical planed surfaces of 2-inch cubical Douglas fir blocks over various exposure periods at four sites.

Site	Period[a] (*months*)	On horizontal faces		On vertical faces	
		Upper	Lower	Side 1	Side 2
Tacoma – 1	2	46	9	28	14
Tacoma – 2	5	2159	278	574	536
Elliott Bay – 1	5	835	77	180	181
Elliott Bay – 2	5	591	68	273	166
Total		3631	432	1055	897
Percentage of total		60.4	7.2	32.4	

[a]Individual strings of 12 blocks each were exposed for 1 month only, the blocks being at 1-ft intervals from 1 to 4 ft below the surface and at 2-ft intervals from 6 to 20 ft. Counts represent the totals on the four 3.6-square-inch planed surfaces of all blocks of the two or five strings used in succession.

Harbor (at one, over a 2-month period; at the other, over 5) and at two locations in Elliott Bay (both over a 5-month period). Combined data indicate 60.4% of the total attack occurred on the upper horizontal surfaces, 7.2% on the lower horizontal surfaces, and 32.4% on the two combined vertical surfaces.

Confirmatory data were obtained by exposing a frame of 12-inch-square plywood panels each month over a 30-month period at site No. 2 in Tacoma Harbor. Over the period, 26,986 *Bankia* attacked the upper panel surfaces and 936 organisms the lower ones, 97 and 3% respectively.

Corresponding observations for *Limnoria* spp. showed the reverse situation. A total of 687 borers attacked the plywood panels over the 30-month period, 97% of the attack on the lower horizontal surface.

VARIATION IN ATTACK WITH DEPTH

The number of *Bankia* attacking blocks immersed in Elliott Bay at depths increasing by 5-ft intervals ranged from less than 100 at mean lower low tide level, and 163 at the 5-ft depth, to more than 800 per ft^2 at the mudline (Fig. 1). Results are averages of monthly counts obtained over a continuous 18-month period which included two falls and two winters.

In a similar experiment over an 18-month period at Alameda, California, limnorial attack was heaviest at the surface, declining sharply to the 15-ft depth, and remaining relatively constant with increasing depth (Fig. 1).

IMMERSION PERIODS

Immersion of duplicate strings of blocks in Vancouver Harbor showed average fresh attack to be declining over 4 consecutive months, as follows.

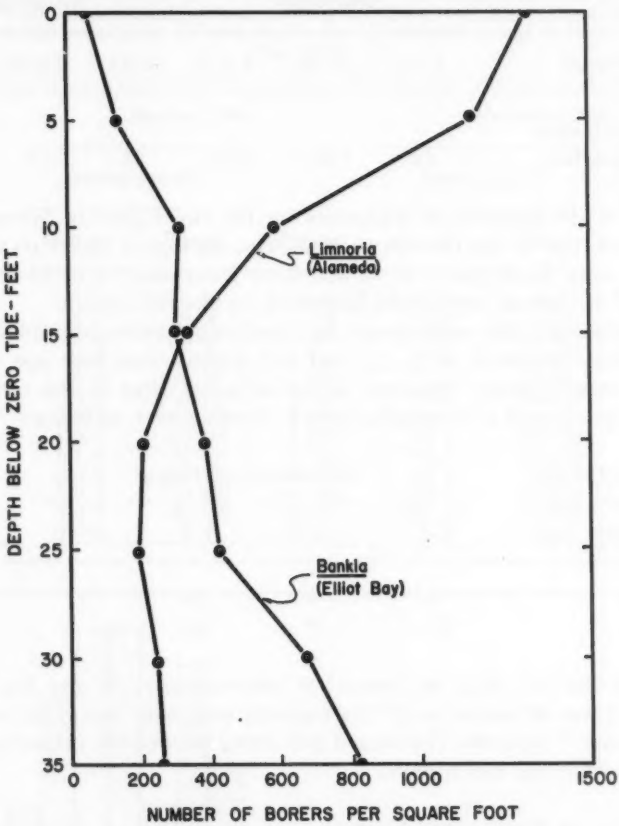

FIG. 1. Attack of bankian and limnorial borers on 2-inch cubical blocks immersed during 18 months at 5-ft intervals from zero tide to the mudline in Elliott Bay, Seattle, and Alameda Harbor, San Francisco. Numbers are the average counts obtained over 18 consecutive months on individual blocks, each immersed for 1 month.

Period	August	September	October	November
Number of borers per square foot	670	363	236	87

When other duplicate strings of blocks were immersed at an adjacent location for the first and second halves of September and November, counts recorded were as follows.

Period	September			November		
	1–15	16–30	Total	1–15	16–30	Total
Number of borers per square foot	32	130	162	28	11	39

The sum of the numbers of organisms for the two halves of September was only 46% of that for the full month. Similarly, the sum of attack on test blocks immersed from November 1 to 15 and from November 16 to 30 was slightly under half of that on test blocks immersed for the full month.

Confirmatory data were sought by classifying *Bankia*, imbedded in blocks after 30 days' exposure at 1-, 2-, and 3-ft depths, into four age categories, duplicate strings being immersed at non-adjacent sites in the same marine area during a period of increasing attack. Results were as follows.

Number of borers per square foot	Estimated age (days)				
	1–3	4–5	6–7	8–30	Total
Site No. 1	6	3	24	27	60
Site No. 2	63	9	45	3	150

Data from the two sites are somewhat contradictory. At site No. 1, where total attack was 60 borers per ft^2 the majority were over 5 days old and almost half were over 7 days old. The second test string showed the majority of borers attacked within the last 3 days.

Reliability of Population Counts

To determine the reproducibility of the block method for recording attack, twelve strings of blocks stapled at 1- and 2-ft intervals to the 20-ft depth were immersed in Vancouver Harbor for the month of October 1963, and 12 others for November 1965 (Table II). Analysis of variance for the 1963 data indicated a standard error of 95 borers per ft^2. The usefulness of this statistic is limited, since the distribution of individual values was non-Gaussian.

In an attempt to improve the normality of the data, individual values were transformed to fractions of the mean for each immersion depth and original counts below 20 borers per ft^2 were excluded. Despite the transformation of the data, the remaining population distribution remained skewed, in a positive manner (Fig. 2). Homogeneity tests of the variances for these transformed data indicated that the remaining population could be pooled as homogeneous. For these transformed hemogoneous data, the standard error was calculated as 0.63 times the mean value for any given depth. For counts below 20 borers per ft^2, the standard error, was greater.

TABLE II. Attack by *Bankia setacea* on the planed surfaces of 2-inch cubical Douglas fir blocks immersed for 1 month at the same location, showing the average and range of organism numbers per square foot and variance for different depths for 12 immersed strings of blocks in each of two years.

Depth (*ft*)	October 1963			November 1965		
	Bankia per ft²			*Bankia* per ft²		
	Average	Range	Variance	Average	Range	Variance
1	3.3	0–20	36	3.6	0–30	86
2	6.7	0–20	49	12.7	0–30	152
3	18.3	0–80	447	15.5	0–30	87
4	23.3	0–50	272	20.0	0–50	240
6	40.0	0–70	500	37.3	0–80	662
8	72.5	30–180	1, 669	65.5	40–130	587
10	113.3	60–210	1, 439	64.5	10–130	1, 707
12	173.3	80–250	5, 889	116.4	40–230	2, 726
14	163.3	60–460	15, 988	122.7	60–220	3, 642
16	230.9	130–480	10, 441	180.9	90–260	2, 541
18	278.3	110–440	11, 464	230.0	60–410	8, 700
20	380.8	120–980	51, 707	247.3	80–420	10, 582

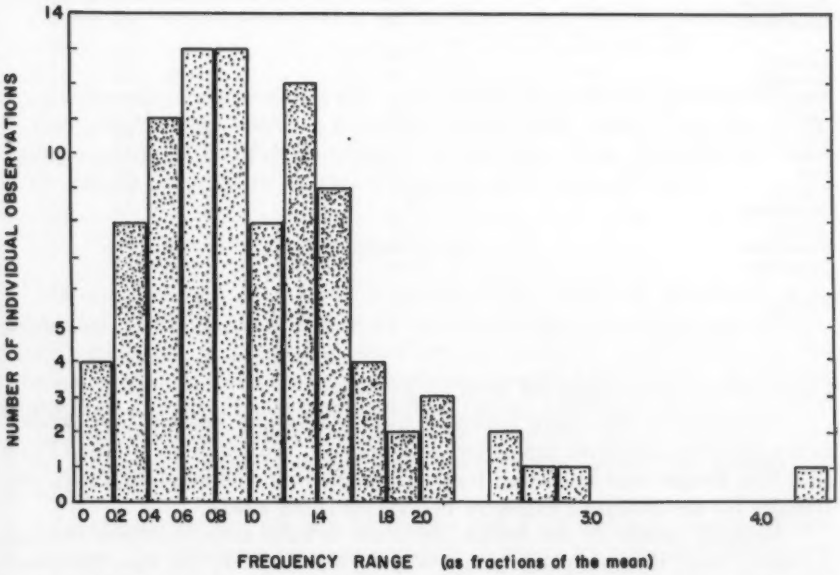

FIG. 2. Distribution about the mean of those bankian counts above 20 borers per ft², obtained after immersion in Vancouver Harbor for the month of October 1963, of 12 strings with 2-inch cubical blocks at 1-ft intervals from 1 to 4 ft below the surface and at 2-ft intervals from 6 to 20 ft. Individual block counts have been transformed to fractions of the average count for each immersion depth.

Comparison of Blocks and Panels

Because of the widespread use elsewhere of Clapp panels, comparative studies were made, over a 19-month period, of counts on single blocks and 1×6×12-inch panels immersed at 20 ft below mean lower low tide in Elliott Bay, Seattle (Table III). In some months, lower counts were recorded on blocks; in others, on panels. For August and September, attack on the cubical blocks was considerably higher than on the panels: 1494 and 1998 per ft² compared with 43 and 28, respectively.

Table III. Numbers of *Bankia setacea* attacking planed surfaces of single 2-inch cubical blocks and 1 × 6 × 12-inch panels of Douglas fir, individually immersed in Elliott Bay for 1-month periods at the 20-ft depth, over an elapsed time of 19 months.

Month	Number of *Bankia* per square foot of planed surface	
	2-inch blocks	6 ×12-inch panels
June	144	273
July	450	409
August	162	70
September	270	3
October	108	256
November	81	7
December	0	75
January	9	28
February	0	13
March	9	Lost
April	45	Lost
May	171	45
June	0	18
July	72	31
August	1494	43
September	1998	28
October	270	64
November	36	6
December	Lost	3
Average	296	81

Comparisons of Attack on Blocks and Logs

Comparative data were collected from blocks and floating logs exposed in the same location over the period September 1, 1963 to June 1, 1964 (Table IV). The blocks were immersed for 1-month periods, the individual logs continually for the indicated exposure periods up to 36 weeks.

Bankian attack on the blocks continued in substantial numbers through January, when the cumulative count was 1070 per ft². On the logs, the count at the dissoconch stage in December was 1100, which, together with the young and older adults, gave a total attack of 1740. During the following 5 months, the total counts on the logs ranged between 1230 and 1910. The average count for the six logs was 1560 *Bankia* per ft².

TABLE IV. Numbers of *Bankia setacea* attacking planed surfaces of individual 2-inch cubical Douglas fir blocks immersed at 1-ft depths for 1-month periods and undersurfaces of floating individual 12-inch diameter hemlock logs immersed for periods from 4–9 months.

			Logs			
			Number of *Bankia* per square foot			
			Size (*mm*)			
Month	Blocks; cumulative[a] no. of punctures per square foot	Exposure periods (*weeks*)	0–4	5–22	23–362[b]	Total
September 1963	50	2				
October	180	6				
November	770	11				
December	970	16	1100	330	310	1740
January 1964	1070	20	720	470	370	1560
February	1080	24	600	160	470	1230
March	1080	28	680	230	590	1500
April	1080	32	560	370	980	1910
May	1080	36	530	130	760	1420

[a]Data were obtained from blocks immersed for monthly periods and are presented cumulatively to facilitate comparison with log data.
[b]Maximum size, encountered only in the log immersed from September to May.

In the logs, about one-half the larvae which attacked and metamorphosed to the juvenile stage failed to reach the young adult stage. Thus, in all the logs an average of 860 *Bankia* matured to the adult stage. Roughly one-third of the adults did not live beyond the young adult stage (22 mm).

DISCUSSION

In monitoring marine-borer populations for programs associated with protection of marine structures or log inventories, early detection and quantitative estimation of population densities are important particularly for the teredine borers, which can cause severe damage within 3 or 4 months. Statistical evaluations (Table II) have not permitted precise assessment of the accuracy of individual measurements because of non-normality of the data, but they have indicated considerable variation, particularly where low counts are involved.

In comparing the efficacy of blocks and 1×6×12-inch panels as test specimens, the difference between numbers of borers on individual blocks and panels (Table III) is not surprising in view of the statistical data. However, the highest individual counts were obtained on the blocks. These higher counts can be explained partly in that four equal surfaces of the blocks were exposed to horizontal water flow during immersion and these four sides were included

in making counts, whereas in the thinner panels only the two opposite flat surfaces were examined. In addition, the upper horizontal surfaces of the blocks received an extremely high attack by teredine borers (Table I), and no horizontal surface was used with the panels. Also, frames containing panels are likely to position themselves so that the flat sides of the panels, which are the surfaces examined, are parallel to water currents and many of the larvae are swept past the wooden surface. Since experimental data demonstrate that blocks are more sensitive than panels in recording high counts, strings of blocks are the more useful in routine monitoring work.

Although the general failure of untreated piling at the mudline suggests that bankian attack is heaviest at this depth, nevertheless, the documentary evidence (Fig. 1) is striking. At the mudline, the average infestation over the 18-month examination period was extremely heavy, more than 800 organisms per ft^2. Population overcrowding would ensure that only a fraction of these organisms would survive to adulthood. Although attack does decline almost linearly with decreasing depth, attack at all depths must be considered heavy. Although the numbers of *Limnoria* attacking per square foot of wood surface were greater than for *Bankia*, the rate of destruction by *Limnoria* would be less; even the extremely heavy attack in surface waters would require several years to decimate a pile.

The limnorial attack below 15 ft (Fig. 1) is of considerable significance since the attack was recorded at Alameda, where the creosote-resistant borer *L. tripunctata* predominates. In this area, pile barriers are used frequently to protect impregnated piles from limnorial attack. Since at no depth did the average monthly attack fall below 200 per ft^2, pile barriers must extend from the mudline to the surface, rather than covering the intertidal area only.

Since borer attack varies with depth, information concerning attack at sub-surface depths is important. *Bankia* larvae are produced mainly at the bottom and spread to the surface in declining numbers. Thus, in those locations which are free of *Bankia* at certain times of the year, surface waters have a considerably longer *Bankia*-free period than those at or near the mudline. By continuous monitoring to depths well below those under protection, the impending rise and fall in teredine attack can be registered at lower depths before these changes are evident in waters nearer the surface. Thus, short-term future attack and potential damage to commercial structures can be predicted with reasonable success. Although early detection of the migration of small numbers of *Limnoria* is not as essential for the protection of commercial structures as it is for *Bankia*, the area of maximum attack is just below mean lower low tide level. Variation in attack with depth does not provide the same warning of impending attack as it does for *Bankia*.

The position and depth of immersion blocks for monitoring programs can be modified to suit individual requirements. For floating structures or log rafts which draw less than 10 ft of water, a useful technique is to immerse blocks to 20 ft below the water surface, at 1-ft intervals to 4 ft and at 2-ft intervals thereafter. Where attack below the 10-ft depth is of importance,

such as in dry-dock protection programs, approximately the same monitoring effort produces similar information by immersing blocks from the surface to the mudline, at 5-ft intervals.

Precise evaluation of low levels of *Bankia* attack in surface waters is critical in the seawater storage of lumber and plywood logs. For this purpose, exposure of large, wooden surfaces in the horizontal plane is desirable, the upper surface being particularly sensitive to bankian attack and the lower surface to limnorial attack. More useful than blocks are the 12-inch-square, 3-ply veneer panels immersed in the horizontal plane. Each panel has an exposed surface 20 times larger than that of a block, i.e. 2 ft^2.

It has been suggested (Zobell, 1946, p. 195) that fresh wood surfaces are less acceptable to marine-borer attack than those immersed for some time. During a period when intensity of attack was declining, reduction of the immersion period from 1 month to half a month materially reduced the recorded rate of attack. Estimating the age of young *Bankia*, imbedded in blocks after 30 days' immersion during a period of increasing attack, did not provide supporting data in one instance, since the technique did not differentiate young adults in the 8- to 30-day age-group. Nevertheless, the evidence indicates a desirable immersion period to be in excess of 2 weeks.

In most commercial situations, significant wood shrinkage begins 60 to 90 days after *Bankia* larval puncture. Thus, immersion periods in excess of 30 days are not practical, since 30 to 45 days are required for immersion, transportation of immersion metarial and its examination, leaving a minimum of 15 days for remedial action in some instances. Thus attack on blocks immersed for 30-day periods was examined to determine how closely data obtained corresponded to cumulative attack in a commercial situation.

Over a 9-month immersion period, block data (Table IV) indicated that fresh attack was moderate to heavy from September through January, ended in February, and was absent from March through April. After immersions from 4–9 months, counts on the six individual log sections ranged from 1230 to 1910 per ft^2 apparently independently of immersion time. Since this variation is compatible with that found in the replicability studies, the data are not in disagreement with the block data which indicated that heavy fresh attack did not occur after January.

Nevertheless, attack on the logs was about 50% higher than the cumulative count obtained with test blocks. Although this difference may be real, it may have resulted in part from statistical variation, as well as from immersion period differences. Immersion depth also differed, the depth of the log undersurfaces depending on the diameter and floatability of individual logs. However, counts obtained with blocks immersed for 1 month corresponded reasonably close to attack on floating logs, and even more closely to the number of adults surviving to the adult stage. The block data (Table IV) also show that some *Bankia* larvae were present in February, a fact which is obscured by statistical variation in comparing data for logs withdrawn from the water at the month ends of January and February.

The data described here have proven useful in designing immersion racks for evaluating impregnants intended to protect wood against marine borers. For example, racks are constructed to that the face of the immersion panel is in the horizontal plane, thereby permitting maximum attack of *Bankia* on the upper surfaces and maximum attack of *Limnoria* on the lower surfaces. To permit maximum attack by the individual borers, panels intended for test against teredine species are immersed at or near the mudline and panels being tested for resistance to limnorial attack are immersed immediately below mean lower low tide level.

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Glycerylphosphorylcholine and Related Compounds in Rainbow Trout Muscle Stored at –4 C[1]

By R. E. E. Jonas and E. Bilinski

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver, B.C.

ABSTRACT

Glycerylphosphorylcholine in rainbow trout (*Salmo gairdneri*) muscle stored at –4 C showed an increase from 36 μmoles/100 g in fresh muscle to 46 μmoles/100 g after 2 weeks. During longer periods of storage an approximately threefold increase in concentration took place, reaching 123 and 105 μmoles/100 g muscle after 9 and 17 weeks. Liberation of free choline was found to take place after 6 weeks of storage. There was very little change in the concentration of choline after 6 weeks storage when the value was approximately 100 μmoles/100 g. The release of free fatty acid during cold storage showed a general trend, which was similar to the formation of glycerylphosphorylcholine, but quantitatively the changes were more pronounced. Free fatty acids amounted to 45 μmoles/100 g in fresh muscle and rose to a plateau of approximately 1200 μmoles/100 g after 9 weeks of storage. The results are discussed in relation to the enzymic activity present in fish muscle.

INTRODUCTION

The occurrence of various types of lecithinase activity in rainbow trout muscle has been investigated previously (Bilinski and Jonas, 1966) and it was shown that the main pathway of lecithin catabolism in trout muscle is via glycerylphosphorylcholine (GPC). The formation of GPC by fish muscle enzymes has also been reported by Yurkowski and Brockerhoff (1965), who have shown that GPC was liberated from lysolecithin under the action of cod muscle lysolecithinase. Although the hydrolysis of phospholipids in fish flesh during cold storage has been investigated rather extensively and various products of hydrolysis have been determined (for review see Lovern and Olley, 1962), the formation of GPC has not previously been studied. Information regarding the occurrence of GPC in fresh fish muscle is also lacking.

In the present paper the amounts of GPC were determined in fresh rainbow trout fillets and in fillets stored at –4 C for periods of up to 17 weeks. In view of their relationship to GPC, choline and free fatty acids (FFA) were also determined.

MATERIALS AND METHODS

Fish

Two-year-old rainbow trout (*Salmo gairdneri*) weighing approximately 200 g were obtained from a commercial hatchery and kept in a freshwater

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aquarium. They were fed New Age complete trout fingerling pellets (J. R. Clark Co., Salt Lake City, Utah). The fish were killed by a blow on the head, bled, and iced. Fillets were prepared without delay, wrapped in cellophane and stored at −4 C.

Total Lipid and FFA

Total lipid was depermined by the method of Bligh and Dyer (1959). Silicic acid chromatography was used to remove phospholipids (Olley and Lovern, 1960) and FFA were titrated in ethanol with 0.05 N KOH using phenolphthalein as indicator.

Phosphorus and Choline in Total Lipid

From an aliquot of the total lipid, phosphorus was determined by King's (1932) method and choline by Glick's (1944) method.

GPC and Free Choline

The method used was essentially that of Schmidt et al. (1952) with only slight modifications. A known weight of fish muscle was cut into small pieces with scissors and homogenized with twice its volume of 10% trichloracetic acid for 2 min and then twice more for 1 min at 1-min intervals all at 0 C – 5 C in an ice bath with an omnimixer. The homogenate was filtered using a Whatman No. 1 filter paper on a Buchner funnel. The trichloracetic acid was removed from the filtrate by extraction with diethyl ether. The filtrate was neutralized to *p*H 7.0 and the volume reduced in a flash evaporator at below 40 C. The solution was adjusted to *p*H 5–6 with acetic acid, and 20% mercuric acetate solution was added (1 g mercuric acetate per 10 g muscle), centrifuged, and filtered. Hydrogen sulphide gas was passed through the solution, then the solution was filtered and the hydrogen sulphide removed by a stream of air under cooling in ice water. One half of the extract was used for the determination of free choline. The other half was heated in a boiling water bath for 20 min in 1 N HCl, under which conditions GPC is hydrolyzed quantitatively to choline and glycerophosphoric acid. The solution was made just alkaline to phenolphthalein with 6 N NaOH. Choline was determined by Glick's (1944) method. The amount of GPC present was calculated from the difference between the choline found after hydrolysis and that in the unhydrolysed portion of the extract. The method is applicable to the study of the liberation of choline and GPC in tissue by enzymic hydrolysis and to the estimation of GPC in fresh tissue. The choline value found in fresh tissue is not indicative of the amounts of free choline, as it may also include compounds such as acetylcholine.

Paper Chromatography

Standard GPC and choline, and aliquots of the muscle extract, treated as above, were chromatographed on Whatman No. 1 filter paper using the one directional descending technique. The solvent systems used were: ethanol –

concentrated ammonia – water (90:5:5, v/v) (Bregoff et al., 1953); *n*-propanol–concentrated ammonia – water (60:30:10, v/v) (Dawson, 1955). The modified Dragendorff reagent (Bilinski, 1964) was used for developing the chromatograms.

RESULTS

The lipid composition of the fresh rainbow trout muscle used for storage experiments is shown in Table I, The muscle contained 0.61% phospholipid, which represented 26% of the total lipid. The choline containing phospholipids accounted for approximately two-thirds of the total phospholipid. As the triglyceride content of trout muscle fluctuates in response to changes in nutritional conditions and seasonal variations, whereas phospholipids remain fairly constant, being an integral part of the cell, the results of the storage experiments were expressed as a percentage of muscle weight rather than as percentage of total lipids.

TABLE I. Lipid composition of rainbow trout muscle.

	Units	Amount
Total lipid	g/100 g muscle	2.33±0.72 (14)[a]
Phospholipid	g/100 g muscle[b]	0.61
Phospholipid	g/100 g total lipid	26.1
Lipid P	μmoles/100 g muscle	786. ±144 (5)
Lipid choline	μmoles/100 g muscle	518. ±87 (4)
Choline containing phospholipid	% of phospholipid (molar ratio)	66

[a]Mean value and standard deviation. The number of determinations is indicated in parentheses. Each determination represents results obtained from one fish.
[b]Calculated from P content × 25.

Data in Table II show the amounts of GPC and FFA in fresh muscle and changes in their concentration during cold storage. Figures on the formation of the free choline during cold storage are also included. GPC was present in fresh rainbow trout muscle at a concentration of 36 μmoles/100 g and it showed a small increase after 2 weeks of storage. During longer periods of storage an approximately threefold increase in concentration of GPC took place, reaching 123 and 105 μmoles/100 g after 9 and 17 weeks respectively. Liberation of free choline was found to take place after 6 weeks of storage. The concentration of free choline remained fairly constant after a storage period of 6 weeks. As shown in Fig. 1, when the combined amounts of GPC plus free choline are plotted against time, there is a linear increase between the 2nd and 9th weeks of storage.

TABLE II. Effect of cold storage at −4 C on GPC, choline, and FFA content of rainbow trout muscle.

Storage time (*weeks*)	μmoles per 100 g muscle		
	GPC	Choline	FFA
Fresh muscle	36.1±11.6 (6)[a]	50.4± 8.5 (6)[b]	44.6±12.5 (8)
2	46.0±10.2 (5)	45.6±22.0 (5)	148.8±41.6 (5)
6	57.4±21.0 (5)	103.6±25.3 (5)	690. ±254. (5)
9	122.8±51.0 (6)	119.3±33.7 (6)	1233. ±295. (6)
17	105.0±48.7 (5)	110.0±24.1 (5)	1166. ±188. (5)

[a]Mean value and standard deviation. The number of determination is indicated in parentheses. Each determination represents results obtained from one fish.
[b]See "Materials and Methods" regarding the free choline in fresh muscle.

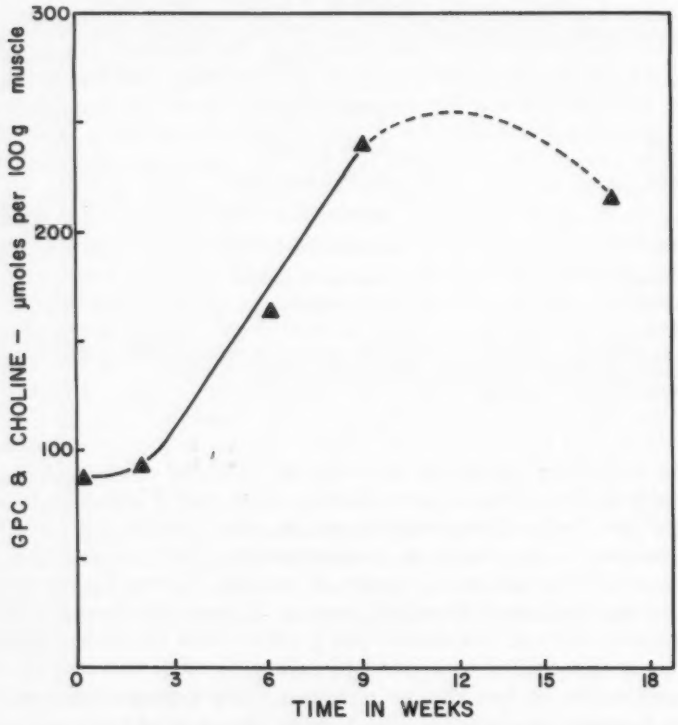

FIG. 1. Changes in the combined amounts of GPC + choline during storage of rainbow trout muscle at −4 C.

In fresh trout muscle FFA were present at a concentration of 45 μmoles/100 g (Table II) and showed some increase during the first 2 weeks of storage. After 6 weeks of storage the concentration had increased to 690 μmoles and in 9 weeks to 1233 μmoles/100 g muscle. There was no evidence for any further increase in FFA. In Fig. 2 the changes in FFA concentration plotted against time also give a linear increase between the 2nd and 9th weeks of storage showing the relationship between the production of GPC and choline and the release of FFA in the hydrolysis of fish phospholipid.

The occurrence of GPC in trout muscle during cold storage was confirmed by paper chromatography. With each of the solvent systems used, a compound giving a positive reaction with the Dragendorff reagent was present at the R_F corresponding to GPC. This compound, after acid hydrolysis, formed a precipitate with ammonium reineckate. The GPC concentration found for fresh fillets (Table II) was not altered when the experimental procedure was modified to the extent of freezing the flesh in liquid nitrogen immediately after the fish was killed.

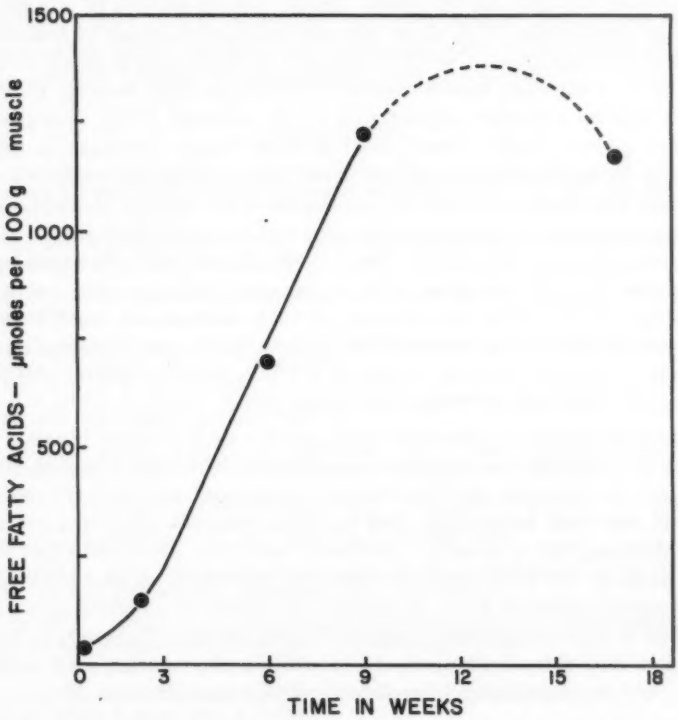

FIG. 2. Changes in the amounts of FFA during storage of rainbow trout muscle at −4 C.

DISCUSSION

The results indicating the formation of GPC during cold storage of trout muscle are consistent with the enzymic activity demonstrated in preparations from fresh rainbow trout muscle (Bilinski and Jonas, 1966). Using tissue slices and homogenates prepared from fresh rainbow trout muscle it was shown that lecithin and lysolecithin were degraded to GPC. Hydrolysis of lecithin to GPC and fatty acids in trout muscle is most probably due to two enzymes: a phospholipase A and a lysophospholipase. It appears very likely that the hydrolysis of lipids in trout muscle during cold storage is due almost entirely to muscle enzymes, as it has been demonstrated with Atlantic cod (*Gadus callarius*) that even when bacteria are freely multiplying, lipid hydrolysis is not detectably influenced by bacterial enzymes (Olley and Lovern, 1960; Lovern and Olley, 1962). It was also shown by these authors that non-enzymic chemical hydrolysis of the lipids does not occur in frozen cod.

The changes in the concentration of GPC and choline in trout muscle during cold storage at −4 C show a trend which is similar to the changes in FFA concentration. A period of linear increase in concentration is noticeable between the 2nd and 9th week. During longer periods of storage neither GPC and choline nor FFA show any well-marked changes in concentration. Quantitatively the amount of GPC released by hydrolysis is small. It does not exceed 20% of the lipid bound choline found in the fresh muscle. This may be partially due to a further degradation of the released GPC, as suggested by formation of free choline during cold storage. Such a process is known to take place in mammalian tissue under the action of phosphodiesterase (Dawson, 1956). The direct liberation of free choline from lecithin under the action of a lecithinase seems unlikely, since this enzyme has not hitherto been demonstrated in animal tissues (Bjørnstad, 1966). A breakdown of choline could possibly be occurring in trout muscle at −4 C in storage. Such a process might be responsible for the fact that the amounts of FFA released are much higher than that expected from the amounts of GPC and choline found. Phospholipids other than lecithin are not a major source of FFA production during phospholipid hydrolysis in fish muscle (Bligh and Scott, 1966).

A general pattern of release of FFA, similar to that found in trout muscle, has been observed before with other species of teleost fish. Olley et al. (1962) found that the amounts of FFA released during cold storage were remarkably similar in the cod, lemon sole, and halibut, rising to a plateau of approximately 300 mg/100 g muscle.[2] Evidence was also presented that in these species most of the FFA arose through the hydrolysis of phospholipids. The relative contribution to FFA production by lipase in rainbow trout muscle, which has a high triglyceride content, is not known. However, it has been established that fatty acid production, even in oily species such as herring, is equivalent to phospholipid breakdown (Olley and Watson, 1962).

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[2]We found 370–350 mg/100 g muscle after 9 and 17 weeks of storage, 300 being used as mean MW of fatty acids.

The results of the present study indicate the presence of GPC in the fresh rainbow trout muscle. The occurrence of GPC in mammalian tissues has been demonstrated in several investigations. It is apparent that GPC is fairly abundant in certain tissues, but only negligible amounts are present in beef skeletal muscle (Schmidt et al., 1952, 1955) and rabbit skeletal muscle (Schmidt et al., 1955). The presence of GPC in fresh heart muscle of frogs and rabbits has been reported by Cantoni and Bernheimer (1947). The occurrence of GPC in tissues may be the result of biosynthetic as well as hydrolytic reactions. The evidence at present available would indicate that GPC occurs in the animal body as an intermediate in the degradation of lecithin (Dawson, 1955; Cornather, 1952) and plays no direct part in biosynthesis of phospholipids (Kennedy, 1957). The breakdown of lysolecithin into GPC may proceed in animal tissue by at least two different pathways: the hydrolysis of lysolecithin to GPC and fatty acid under the action of lysophospholipase and a dismutation of two molecules of lysolecithin with the formation of GPC and lecithin (Erbland and Marinetti, 1965).

The data obtained by us on phospholipid composition of rainbow trout muscle agree with the findings reported by others. Gray and McFarlane (1961) reported 25.4 and 27.8 mg (819 and 891 μmoles) lipid P per 100 g of trout muscle. By means of silicic acid chromatography they found that 68% of the lipid P was present in phospholipid containing choline.

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Returns of Hatchery-Reared Lake Trout in Southern Lake Superior, 1955–62[1]

By Richard L. Pycha[2]

U.S. Bureau of Commercial Fisheries
Biological Laboratory, Ann Arbor, Michigan

AND

George R. King

Wisconsin Conservation Department
Bayfield, Wisconsin

ABSTRACT

Experimental plantings of fin-clipped lake trout (*Salvelinus namaycush*) of various ages have been made in southern Lake Superior since 1952. The catch of planted lake trout by the commercial fishery was used to measure the success of stocking. Estimates of total returns were based on samples of 8.2 to 21.2% of the Wisconsin catches in 1955–62 and 36.4 to 46.8% of the Michigan catches in 1959–62. Marked lake trout were assigned to various plantings according to the fin mark, length of fish, readings of age from scales, and locations of capture.

Estimated returns from Wisconsin plantings of 9-month-old lake trout varied from 2.1 to 6.4%. Wisconsin releases of 16-month-old fish yielded returns of 5.7 to 37.3%. Returns from plantings in Michigan were 0.3% for 9-month-old fish and 1.5 to 3.8% for 16-month-old fish. The ratios of the returns from plantings of 16-month-old fish to those of 9-month-old fish were 3.9:1 for Wisconsin releases and 6.7:1 for Michigan releases. Lake trout planted in Wisconsin generally survived better than those released in Michigan. Returns from fish planted at ages of 17 to 40 months in Michigan in 1958–59 were far greater than those from 16-month-old fish released in Michigan, but were not as high as from the most successful plantings of 16-month-old fish in Wisconsin. Lake trout released in 1960 from shore and from boats survived equally well.

Average size of lake trout at release was more closely related to survival than any other factor. All except 1 of 10 highly successful releases were of fish larger than 22 per lb, and all 6 poor releases were of fish smaller than 34 per lb. Fish that ran 22 to 33 per lb survived only moderately well. A size of 17 to 20 fish per lb appears to be close to optimum.

Abundance of native lake trout, American smelt (*Osmerus mordax*), and sea lampreys (*Petromyzon marinus*) all appeared to be unrelated to stocking success. The only apparent method of rapidly improving the success of stocking is to rear all lake trout to an average size of 17 to 20 per lb.

INTRODUCTION

The sea lamprey (*Petromyzon marinus*) caused the near extinction of lake trout (*Salvelinus namaycush*) in Lakes Huron (Fry, 1953) and Michigan (Eschmeyer, 1957) and severely reduced the stocks in Lake Superior. Rehabilitation

[1]Received for publication August 24, 1966.
[2]Present address: Biological Station, Ashland, Wisconsin.

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of lake trout stocks will depend on control of the sea lamprey, but rapid recovery also requires large-scale stocking. High survival of planted fish is needed because rearing facilities are limited in relation to anticipated requirements for hatchery-reared fish.

Before the 1930's, millions of lake trout fry, hatched from eggs taken from native fish, were planted annually in the upper Great Lakes. Fry stocking was gradually abandoned during the 1930's because the practice appeared to be ineffective (Dymond, 1957). In 1944–46 experimental plantings of 9-month-old fingerling lake trout were made in Lake Michigan, but returns ranged only from 0.06 to 0.67% (Cable, 1956). Sea lamprey depredations and collapse of the lake trout fishery contributed to an unknown degree to the low returns. In 1952–57 experimental releases of both fin-clipped and tagged hatchery-reared lake trout were made in Lake Superior to determine the best age for planting (Buettner, 1961). On the basis of these experiments, the age of 16 to 18 months appeared to be most economical.

The Michigan Department of Conservation undertook establishment of a lake trout brood stock in 1949. After successfully rearing the 1949 year-class for 4 years, the Department expanded the brood stocks to provide enough eggs by 1965 for annual plantings of 5.5 million yearling lake trout.

The Great Lakes Fishery Commission, through its contracting agents, the Fisheries Research Board of Canada and the U.S. Bureau of Commercial Fisheries, initiated control of the sea lamprey in Lake Superior in 1958 with a toxicant that selectively destroyed sea lamprey larvae in tributary streams. The Commission encouraged all governmental agencies to increase stocking of yearling lake trout and to mark all planted fish by clipping fins. By 1962 spawning runs of sea lampreys in streams tributary to Lake Superior had been reduced roughly 80 to 85% and plantings of yearling lake trout had been increased to nearly 2 million annually. To aid the rapid recovery of spawning stocks, the commercial lake trout fishery was closed in mid 1962 to all but the minimum amount of fishing necessary for biological sampling.

Although hatchery-reared fish have contributed substantially to the abundance of lake trout in Lake Superior in recent years (over 50% of all legal lake trout caught in U.S. waters in 1964 and many spawning fish in 1963–64 were fin-clipped), the only published data on the success of stocking were some incomplete estimates of the returns from various plantings given in a progress report on lake trout studies (Great Lakes Fishery Commission, 1961). The purposes of this report are to present estimates of returns from various plantings to the commercial fishery, to evaluate the relative success of certain experimental plantings, and to examine factors related to survival of hatchery-reared lake trout in Lake Superior.

The total returns of fin-clipped lake trout in the commercial fishery are the best available measure of the "success" of stocking. Because planted lake trout in Lake Superior were not fully vulnerable to commercial gear until 3 to 5 years after release, these data do not permit an actual assessment of survival even though rates of return are, obviously, related to survival. During the period

covered by this study, sea lampreys competed with the commercial fishery for lake trout of vulnerable size and destroyed an unknown number. Fishing intensity was high enough, however, that large numbers of planted fish were taken in the fishery. Because rates of return are dependent on fishing effort, no estimates of total returns are given for the years since the season closure (1962). Data for 1962 are included because fishing effort increased that spring and raised the annual production to near the 1961 level in spite of the midyear closure.

MATERIALS AND METHODS

Field Procedures for Collection of Data

The Bureau of Commercial Fisheries and the Wisconsin Conservation Department took part in the intensive sampling of the commercial catches of lake trout in Lake Superior in 1959–62. The Bureau employed nine commercial fishermen in Michigan and one in Wisconsin to record data from their catches. Wisconsin Conservation Department employees accompanied most of the lake trout fishermen in Wisconsin, on a rotating schedule, to check their catches. All lake trout caught (except live undersized fish, which were released in conformity with usual fishing practices) were examined for clipped fins and sea lamprey wounds or scars. Data on total length (inches) and missing fins were recorded for all marked fish. Either entire catches or net-run samples of fish were measured and scale samples were taken periodically. Records were kept on location, depth of water, amount of gear lifted, and total number and weight of legal-sized[3] lake trout caught. Lake trout fishing was done mainly with large-mesh ($4\frac{1}{2}$–$4\frac{3}{4}$ inch, stretched measure) nylon gill nets, but a small part of the Wisconsin production came from pound nets from which all undersized fish were released. Samples were obtained from nearly all major fishing grounds in Michigan and Wisconsin (Fig. 1). In 1959–60 several additional commercial lake trout fishermen in Michigan voluntarily kept records from their catches. These data agreed closely with those collected in the same sampling areas by Bureau employees. The records obtained from all sampling areas of Fig. 1 were, therefore, considered representative of the production within the areas.

Complete records kept voluntarily by three commercial fishermen in Wisconsin in 1955–58 made possible an estimate of the total return of fin-clipped fish in Wisconsin for those years. A comparison of 1959–62 records of these fishermen with the entire Wisconsin samples indicated that their catches were representative of the entire Wisconsin production.

One Michigan fisherman at West Entry provided complete records of lake trout caught in small-mesh ($2\frac{1}{2}$–$2\frac{3}{4}$ inch gill nets fished for chubs (*Coregonus* [*Leucichthys*] spp.)), and Department personnel accompanied Wisconsin chub fishermen to obtain samples from small-mesh nets in 1959–60.

[3]In Michigan the minimum size was $1\frac{1}{4}$ lb, dressed weight; in Wisconsin the minimum size was 17 inches total length. The two minimums were practically the same.

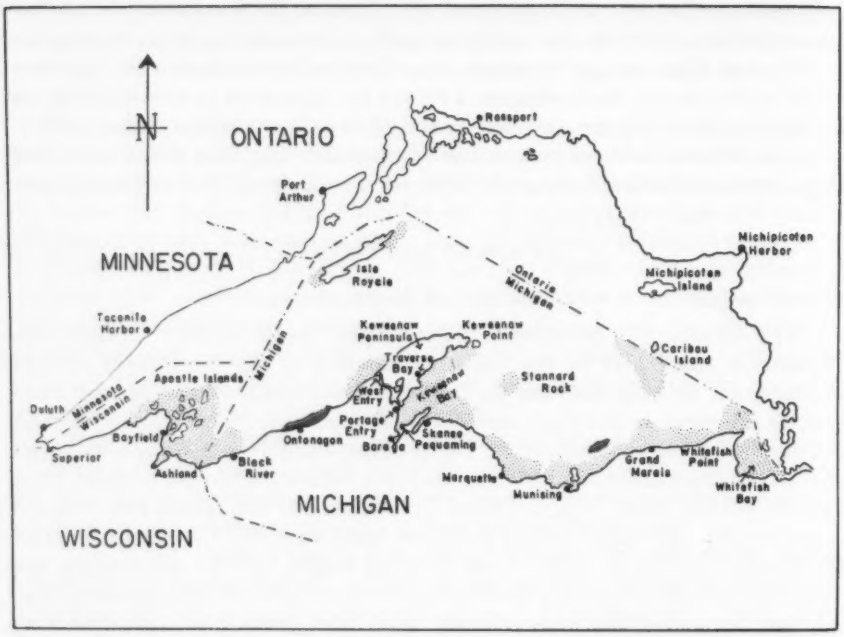

FIG. 1. Major grounds fished for lake trout in Wisconsin and Michigan waters of Lake Superior, 1959–62. Samples were obtained from stippled areas in 2 or more years; no samples were obtained from crosshatched areas.

Wisconsin samples from large-mesh gear in 1955–58 amounted to 147,780 lb of legal-sized lake trout (Table I) and included 4077 fin-clipped fish. The 1959–62 samples totaled 86,543 lb and contained 11,775 fin-clipped fish. These samples constituted 8.2 to 11.2% (average, 9.4) of the annual Wisconsin production of lake trout in 1955–58 and 13.6 to 21.2% (average, 16.8) of the totals in 1959–62. Samples from large-mesh nets in Michigan waters in 1959–62 totaled 439,687 lb of legal-sized lake trout and 11,375 fin-clipped fish, and made up 36.4 to 46.8% (average, 39.1) of the production of lake trout in the areas for which estimates of returns were made. Offshore grounds near Isle Royale and Stannard Rock contributed only about 5% of the Michigan catches in 1959–62; samples from these areas contained so few fin-clipped fish that no estimates of returns were made.

The samples from small-mesh gear constituted 16.9% of all small-mesh nets fished in Wisconsin and at West Entry in 1959, and 8.0% of the total in 1960 (nearly all lake trout taken in small-mesh nets were undersized and were not reported in production statistics). Samples from small-mesh nets fished in other years and areas were inadequate for the estimation of returns of marked fish.

TABLE I. Production of lake trout (pounds, dressed weight), sample size, and percentage of production sampled in different areas, Lake Superior, 1955–62. (Areas listed are those for which estimates of returns were calculated.)

Year and item	Wisconsin	Michigan							
		Black River-Ontonagon	West Entry	Keweenaw Bay	Marquette	Munising-Grand Marais	Caribou Island	Whitefish Point and Bay	All Michigan areas
1955									
Production	552,690								
Sample	48,746								
Percentage	8.8								
1956									
Production	479,259								
Sample	53,582								
Percentage	11.2								
1957									
Production	286,765								
Sample	24,220								
Percentage	8.4								
1958									
Production	258,052								
Sample	21,232								
Percentage	8.2								
1959									
Production	131,966	14,951	8,311	225,553	61,359	227,385	52,429		589,988
Sample	24,694	7,292	8,311	67,035	24,095	55,602	52,429		214,764
Percentage	13.6	48.4	100.0	29.7	39.3	24.5	100.0		36.4

(*Coutinued*)

TABLE I. Production of lake trout (pounds, dressed weight), sample size, and percentage of production sampled in different areas, Lake Superior, 1955–62. (Areas listed are those for which estimates of returns were calculated.) — (*Concluded*)

Year and item	Wisconsin	Michigan							All Michigan areas
		Black River-Ontonagon	West Entry	Keweenaw Bay	Marquette	Munising-Grand Marais	Caribou Island	Whitefish Point and Bay	
1960									
Production	108,649	6,694	4,742	108,104	21,791	63,666	50,450		255,447
Sample	16,900	1,771	4,742	29,289	5,570	12,146	50,450		103,968
Percentage	15.6	26.5	100.0	27.1	25.6	19.1	100.0		40.7
1961									
Production	102,526	5,366	8,373	69,705	20,010	36,267		14,457	154,178
Sample	21,713	1,224	6,908	23,827	7,231	13,696		9,203	62,089
Percentage	21.2	22.8	82.5	29.3	27.7	37.8		63.7	40.3
1962									
Production	121,202	6,365	4,259	71,328	13,634	20,625		9,629	125,840
Sample	23,236	1,669	4,061	30,518	5,077	11,629		5,912	58,866
Percentage	19.2	26.2	95.4	42.8	37.2	56.4		61.4	46.8
Total 1955–58									
Production	1,576,766								
Sample	147,780								
Percentage	9.4								
Total 1959–62									
Production	514,343	33,376	24,685	474,690	116,794	347,943	102,879	24,086	1,125,453
Sample	86,543	11,956	24,022	150,669	41,973	93,073	102,879	15,115	439,687
Percentage	16.8	35.8	93.5	31.7	35.9	26.7	100.0	62.8	39.1

Estimation of Returns

Monthly production statistics from large-mesh gear by port were obtained from records of the Wisconsin Conservation Department and the Michigan Department of Conservation. Statistics on fishing effort for small-mesh gear were obtained from the Wisconsin Conservation Department. All production from large-mesh nets was assigned to the corresponding sampling areas (Fig. 1). Estimates of total catches of fin-clipped fish were made by 1-month periods for Wisconsin, but samples were not available for all months at all ports in Michigan. The percentage and size composition of the various groups of marked fish in the Michigan catches changed little within the periods of January–July, August–September, and November–December (the lake trout fishing season was closed during most of October). Estimates for Michigan waters were made for these sampling periods.

Returns were assigned to the various plantings on the basis of fin mark, length of fish, location of recapture, age of fish, or various combinations of these criteria. Ages of a total of 2192 fish were determined from plastic impressions of scales. Because fish from some of the early plantings could not be identified as to year of release, some groups planted in successive years at one location were combined and treated as single releases. A few groups were separated on the basis of location of capture in relation to the planting sites. These separations were based on known movements of planted lake trout (Pycha et al., 1965).

Estimates of total catches of fin-clipped lake trout (Table II) were made by simple proportion. The ratio of the pounds of legal fish in a sample from large-mesh nets to the total production of the sampling area was multiplied by the number of fin-clipped lake trout, both legal and undersized, from each planting in the sample to yield an estimate of the total catch from each release in that gear. No samples were taken at Ontonagon, but estimates of returns from that port were based on the assumption that the percentage of marked fish from each release in the catch was the mean of the percentages at Black River and West Entry (Fig. 1). Estimates of returns in small-mesh nets were made in a similar manner, but were based on the ratio of the amount of gear used to take the sampled catches to the total gear fished. The estimate of returns in 1958 from the 1955 Wisconsin spring release was based on the assumption that the ratio between returns per unit of gear in small- and large-mesh nets was the same for this group in 1958 as for the 1956 Wisconsin release in 1959.

Sources of Bias or Error

No estimates of returns were made for lake trout planted in Wisconsin or Michigan that moved into Minnesota or Ontario waters. The amount of underestimation caused by this movement is unknown, but limited data suggest it should be small except for releases in Whitefish Bay (Pycha et al., 1965).

Regeneration of fins or failure to recognize clipped fins could cause underestimates of returns. Although considerable regeneration can occur (Shetter, 1951), about 97% of all the undersized lake trout caught in inshore waters of

TABLE II. Estimated numbers of lake trout recovered from various 1952–56 Wisconsin plantings by the commercial fisheries of Wisconsin in 1955–62, and Michigan in 1959–62.

Season and year of release	Age at release (*months*)	Number planted (*thousands*)	Gear[a]	1955	1956	1957	1958	1959	1960	1961	1962	Total returns all gears: Number	Total returns all gears: Percentage	Weighted average percentage
Fall 1952–53	9	278.0	L	254	310	1,273	938	2,003	673	130	110	5,782	2.1	3.5
			S					65	26					
Fall 1954	9	142.3	L	64	80	460	1,486	4,063	2,130	280	279	9,084	6.4	
			S					167	55					
Spring 1952–54	16	284.3	L	1,625	3,484	4,753	2,604	2,785	914	163	18	16,346	5.7	15.2
			S					0	0					
Spring 1955	16	102.8	L	197	791	3,880	7,431	18,030	4,943	670	356	38,311	37.3	
			S				1,538	390	85					
Spring 1956	16	200.7	L		0	682	879	3,871	10,837	9,854	7,003	34,989	17.4	
			S					1,203	660					

[a]L = large-mesh, 4½–4¾ inch mesh gill nets and pound nets; S = small-mesh, 2½–2¾ inch mesh gill nets.

Wisconsin and Michigan in 1963–64 and over 99% of the legal lake trout caught in Whitefish Bay in 1964 had clipped fins. Such high percentages indicate that probably neither fin regeneration nor careless observation was a serious source of bias.

Because data were lacking on returns from Michigan waters before 1959, estimates of total returns from Michigan plantings of 1952–55 were serious underestimates and cannot be compared directly with totals for Wisconsin plantings of the same years. The lack of sampling in Michigan before 1959 also caused underestimates of returns from Wisconsin releases made in 1952–56 because many Wisconsin fish moved gradually eastward into Michigan waters (Pycha et al., 1965). The error was greatest for the earliest releases, but was probably not large for the 1955–56 plantings. The 1956 group made little contribution to Michigan catches until 1960, and it seems probable that the 1955 group contributed little in Michigan before 1959.

Fish planted in Wisconsin and at Marquette in the fall of 1954 and spring of 1955 were assigned to their respective releases according to whether they were captured west or east of the Keweenaw Peninsula (Fig. 1). This separation gave a slight bias toward underestimation for the Wisconsin groups and overestimation for the Marquette groups.

Differences in amount of gear fished per unit area of "lake trout water" in Wisconsin and Michigan could affect total returns from plantings made in these states. Fishing intensity per unit area of water less than 40 fath (where most lake trout were caught) was, however, about equal in the two states in 1959–62 and was not greatly different in 1957–58. Differences in fishing intensity, therefore, should have had little effect on the apparent relative success of plantings in the two states.

RETURNS OF MARKED LAKE TROUT

Returns from Wisconsin Plantings of 1952–56

Fin-clipped lake trout were planted in Wisconsin in 1952–56 to compare the survival of 9-month-old fingerlings released in the fall and 16-month-old yearlings released in the spring. All fish were planted among the Apostle Islands from boats.

Lake trout planted in Wisconsin as yearlings survived better than fish of the same year-classes planted as fingerlings. Percentage returns of fingerlings varied from 2.1 for the combined 1952–53 releases to 6.4 for the 1954 group and averaged 3.5 (Table II). Returns from releases of yearlings ranged from 5.7% for the combined 1952–54 plants to 37.3% for the 1955 group and averaged 15.2% for all releases.

Although the 1955 and 1956 releases of yearlings yielded similar numbers of fish to the fishery (Table II), the percentage returns (1955, 37.3%; 1956, 17.4%) were very different and their histories in the fishery were dissimilar. The 1955 group came into the fishery strongly in the fall of 1958, contributed over 18,000 fish in 1959, and then declined to a return of about 5000 in 1960

and 670 in 1961. The 1956 group came into the fishery in the fall of 1959, contributed about 11,000 fish in 1960 and nearly 10,000 in 1961, and still yielded 7000 in 1962. Fishing effort declined about 23% from 1959 to 1960 and nearly 10% from 1960 to 1961. Also, abundance of sea lampreys fell drastically in mid 1961 and remained low thereafter. The decline in fishing intensity undoubtedly reduced fishing mortality of the 1956 group and the additional year of high sea lamprey predation experienced by the 1955 fish probably caused much higher natural mortality than for the 1956 fish. The incidence of lamprey wounds and scars from the fall of 1959 to the spring of 1961 was similar to that in South Bay, Lake Huron, during 1948–51 (Fry, 1953), when natural mortality of lake trout rose from 25 to about 70%. The effect of these factors of mortality should have resulted in a higher percentage return for the 1956 release, but the early returns suggest that the 1955 group survived better in the first year after release and established an advantage that held until the sixth year.

Returns from Michigan Plantings of 1952–55

Fin-clipped lake trout were planted from boats off Marquette, Michigan, in 1952–55, but no data on returns were available until 1959. Comparisons of returns in 1959–62 from plantings made in 1952–55 show that releases made in Wisconsin yielded much higher returns that those made in Michigan (Table III). The 9-month-old fish planted among the Apostle Islands yielded nearly 8 times as high a return in 1959–62 as the 9-month-old fish planted at Marquette (2.3% compared with 0.3%). The 16-month-old fish released in 1953–54 at Marquette yielded a slightly higher return than the Apostle Islands releases of 1952–54, but these groups are not comparable because they covered different

Table III. Estimated returns of lake trout planted in Wisconsin and Michigan in 1952–56, taken in large-mesh gill nets in 1959–62.

Year and location of release	Age at planting (*months*)	Number planted 1952–56	Returns 1959–62	
			Number	Percentage
1952–54				
Apostle Islands	9	420,300	9,688	2.3
Marquette	9	325,700	949	0.3
Apostle Islands	16	284,300	3,880	1.4
1953–54				
Marquette	16	203,800	2,974	1.5
1955				
Apostle Islands	16	102,800	23,999	23.3
Marquette	16	60,700	2,312	3.8
1956				
Apostle Islands	16	200,700	31,565	15.7

periods. Of the two plantings of 16-month-old fish in 1955, the Apostle Islands release yielded slightly more than 6 times the percentage return of the Marquette release (23.3% compared with 3.8%), even though some of the returns assigned to Marquette were probably of Wisconsin origin.

Although the possibility exists that fish planted at Marquette grew faster than Wisconsin fish and came through the fishery before sampling was begun in Michigan, records kept by one fisherman at Marquette in 1958–59 confirmed that fewer marked fish were caught in 1958 than in 1959. The 1959–62 returns, therefore, appear to reflect lower survival of the groups planted in Michigan.

Comparison of Survival of Yearlings and Fingerlings

Previous comparisons of spring and fall plantings indicated that yearlings survived roughly 9 to 10 times as well as fingerlings (Buettner, 1961). The more extensive data of the present study show that the advantage for spring-planted yearlings was not as great as previously reported. The average returns from fish of the 1952–54 year-classes released in Wisconsin in 1952–55 (Table II) were 3.5% for fingerlings and 13.8% for yearlings[4] — a ratio of 3.9:1 in favor of the yearlings. The data from releases off Marquette in the same years (Table III) show a 6.7:1 ratio in favor of yearlings. Webster et al. (1959) reported similar results for yearling and fingerling lake trout released in Cayuga Lake, New York, where yearlings survived 3.94 times as well as fingerlings. They concluded that, on a cost basis, planting fingerlings was as efficient as planting yearlings. Their yearlings were, however, larger in comparison with their fingerlings than was true for the Lake Superior fish. The ratio of costs of planting yearlings and fingerlings would be lower for Lake Superior.

"Success" of Lake Trout Stocking

The 1955 release which had a total return of 37.3% (Table II) probably provided a measure of nearly the maximum return from lake trout stocking. Natural mortality certainly accounted for some loss during the 4 to 5 years before these fish reached full vulnerability to the fishery, but little is known about natural mortality of lake trout — especially at the ages below vulnerability to a fishery. Annual natural mortality rates (n) that have been reported ranged from 22 to 70% for lake trout from various lakes and nearly all values were below 50% (Webster et al., 1959; Ricker, 1958).

A series of simple models with various constant values of n applied to the 1955 Wisconsin release showed that the estimated return could not have been attained if n exceeded 20%. The apparent differences in total annual mortalities of the 1955 and 1956 releases under conditions of high and low sea lamprey predation indicate that natural mortality due to lamprey predation was very high on the 1955 release after the fish reached age V. If losses to lamprey predation equaled fishing mortality after age V (in 1959–61), n could not have

[4]The return from the 1956 release was not included in this average because no corresponding release of fingerlings was made in the fall of 1955.

exceeded 10% in the years before age V. The incidence of lamprey wounds on lake trout in Wisconsin (and presumably natural mortality) was somewhat lower in the spring of 1959 than in the remainder of the 1959–61 period, but even if losses to lampreys were only half as great as fishing mortality, n could not have exceeded 15%. Had there been any large mortality of these fish due to handling during release or to failure of the fish to adjust from hatchery to lake conditions, too few fish would have survived to age V to yield the return actually obtained unless n was practically nil prior to age V or fishing mortality was extremely high after age V. For example, if 25% of the fish that were planted died soon after release and n was only 10% after this initial mortality, the 1959 return would represent a fishing mortality rate (m) of nearly 48% that year. If, as seems probable, n rose much higher than 10% in 1959 because of high lamprey predation, the 1959 return would represent an improbably high value of m. The fact that fishing effort had declined to only about 60% of normal by 1959 makes such values of m appear nearly impossible. Both initial mortality after release and natural mortality in the early years of life must, therefore, have been very low.

Even if initial mortality was nil and n was only 10%, the 1959 return represents a value of m high enough (33%) to cast some doubt on the validity of the estimated return. More recent Wisconsin releases have, however, also produced surprisingly high returns despite an extreme reduction in fishing effort since mid 1962. During 1964, when fishing effort was only about one-tenth as high as in 1959, approximately 3% of the 1960 release was captured by the fishery and checked by Department or Bureau employees. Had the fishery been operating at the 1959 level of intensity in 1964, the percentage return from the 1960 release might have been as high as from the 1955 release and could possibly have been higher. The estimate of the 1959 return, therefore, appears to be reasonable.

The high return from the 1955 Wisconsin release appears to have resulted from low natural mortality at all ages below V and from a high rate of exploitation.

Returns from Plantings Made in 1958–60

All lake trout planted in Lake Superior since the spring of 1958 were fin-clipped yearlings or older (no fin-clipped fish were planted in 1957), and were partially vulnerable to the gear in 1959–62. Although total returns from the 1958–60 releases are not comparable to totals for the 1952–56 plantings, useful comparisons can be made for fish of comparable ages or years at large.

Marked lake trout were released at five locations in Wisconsin and Michigan in 1958: among the Apostle Islands; off Marquette, Munising, and Grand Marais; and in Whitefish Bay. Returns from fish planted at Munising and Grand Marais could not be separated and were, therefore, combined (Table IV). The fish planted in Whitefish Bay were 17 to 21 months old at release; all others were 16 months old.

TABLE IV. Estimated returns of lake trout planted in Wisconsin and Michigan in 1958–60, taken in large-mesh gill nets in 1959–62.

Year and location of release	Age at release (*months*)	Number planted	Number returned					Percentage return 1959–62
			1959	1960	1961	1962	Total	
1958								
Apostle Islands	16	184,000	436	553	2,696	4,022	7,707	4.2
Marquette	16	60,000	29	292	844	326	1,491	2.5
Munising and Grand Marais	16	200,000	125	171	889	850	2,035	1.0
Whitefish Bay	17–21	38,000			1,316	974	2,290	6.0
1959								
Apostle Islands	16	151,100	0	162	482	1,553	2,197	1.5
Baraga	26	27,100	22	237	774	1,424	2,457	9.1
Marquette	26	13,400	77	457	707	617	1,858	13.9
Marquette	40	2,900	10	406	224	96	736	25.4
1960								
Apostle Islands	16	161,400		14	819	1,165	1,998	1.2
Pequaming	16	131,700		0	88	489	577	0.4
Baraga	16	128,800		0	107	491	598	0.5
Munising	16	132,600		0	32	491	523	0.4

Returns in large-mesh nets from the 1958 plantings (Table IV) were greater for fish released in the Apostle Islands region (4.2%) than at Marquette (2.5%). The large planting at Munising and Grand Marais the same year yielded a return of only 1.0%. The Whitefish Bay releases yielded the highest return (6.0%) of any release in 1958, and this figure may be a serious underestimate because substantial numbers from this group, taken in large-mesh nets in Ontario waters of Whitefish Bay, were not included in the estimated return. Date were too limited to estimate the return in small-mesh nets, but the capture of 241 fish of this group in three lifts of chub nets in Whitefish Bay in 1961 suggests that the return was large.

The only yearling lake trout planted in 1959 were released in the Apostle Islands region; the three releases in Michigan were of 26- and 40-month-old fish (Table IV). The lower percentage return from the Wisconsin release (1.5) than returns at comparable ages from the 1955, 1956, and 1958 Wisconsin releases (11.9, 2.7, and 2.0%, respectively) suggests only moderate success. The three groups of older fish planted in Michigan, however, yielded the highest returns of any releases in that state (9.1 to 25.4%). Because the 40-month-old fish were vulnerable to large-mesh nets 1 to 2 years before the 26-month-old fish, the return from the older group was more nearly complete. The actual survival of fish of these three releases may have been similar.

The early returns from fish planted in 1960 (Table IV) were very incomplete, but are useful for evaluation of planting methods. All releases before

1960 were made from boats, in water 5–25 fath deep (the depths preferred by native yearling lake trout). In 1960, the plantings off Pequaming in Keweenaw Bay and off Munising were made from boats, but those at Baraga and the Apostle Islands were made directly from shore. This sites at Baraga and the Apostle Islands were within 1 or 2 miles of 15-fath depths offshore and were areas without large numbers of predacious fish. The releases at Baraga (from shore) and off Pequaming (from boats) were made as a controlled experiment — they were at locations about 6 miles apart, the fish were a single hatchery lot divided into groups of identical size and age, and poposite ventral fins were clipped for identification.

The return in the third year at large for the 1960 Wisconsin fish planted from shore (0.7%) was over twice the return at the same age from the 1958 (0.3%) or 1959 (0.3%) Wisconsin releases from boats, and was exceeded only by that of the 1955 Wisconsin plant (3.8%). The fish released at Baraga and off Pequaming yielded closely similar returns in the third year at large, and the group planted by boat near Munising also had a similar percentage return (in 1963–64, however, the Munising group yielded many more returns than the other 1960 Michigan plants and may have been nearly as successful as the Wisconsin fish). Of the 1960 plantings, the group released in Wisconsin again produced greater percentage returns than those released in Michigan (Table IV).

Data from the 1960 plantings indicate that planting from selected shore sites was as efficient as planting from boats. Shore planting is far more economical than boat planting and involves less handling of the fish. The fish released from shore disperse to preferred depths within a few hours (Pycha et al., 1965).

FACTORS AFFECTING SURVIVAL OF PLANTED LAKE TROUT

Although the plantings considered in this study were not strictly comparable (because of differences in locations, seasons, and years of release, sources of eggs, rearing stations, diets, and size and age of fish at release), the percentage returns from the releases were more closely related to size of the fish at release than to any other factor. Records of the plantings listed in Tables II–IV, and of five more recent releases, provided information on the relation of size at planting to subsequent "success". Because data collected after closure of the fishery in 1962 were not comparable to those obtained in earlier years, precise quantitative ratings of success could not be made. Comparisons of catches per unit of effort at various ages in both experimental and commercial gear, in addition to the information on total returns, formed the basis of ratings (partly subjective) of the success of various plantings made in 1952–62 (Table V).

Lake trout in all but 1 of 10 plantings that were rated "excellent" or "very good" averaged 21 per lb or larger at release. Fish of releases rated "poor" or "very poor" averaged 35 per lb or smaller. Those of "good" or "fair" releases were in the range of 22 to 33 per lb. The relation between size at release and success (Fig. 2) shows that sizes of 17 to 20 per lb are close to optimum for stocking lake trout.

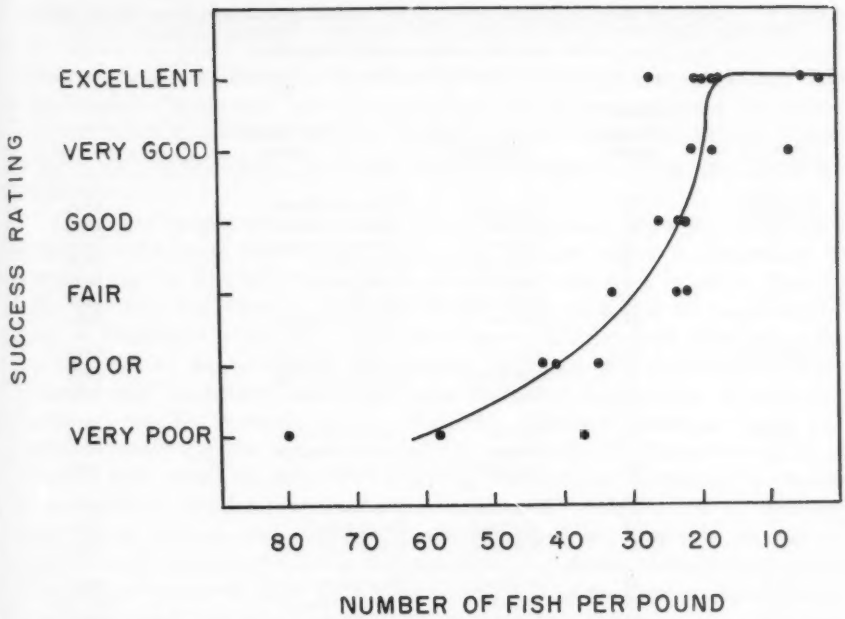

FIG. 2. The relation between size of lake trout at release and success of stocking. (Curve drawn by inspection.)

Although releases of yearlings in Wisconsin prior to 1960 were consistently more successful than those made in Michigan, the available evidence does not indicate that the cause was a more favorable environment in Wisconsin. In 1961, equal numbers of yearlings reared at the Salmo Hatchery in Wisconsin and the National Fish Hatchery in Michigan were released in Wisconsin (before 1961 all fish reared in Michigan were released in Michigan). About one-third of the Salmo Hatchery fish came from eggs taken from native Apostle Islands fish, but all other eggs came from brood stock held in Michigan. Fish from the two hatcheries were marked separately and were released in equal numbers at several shore sites (the National Hatchery fish were held 2 to 3 days in Wisconsin before release). The National Hatchery fish were larger (22 per lb) than the Salmo Hatchery fish (27 per lb) and the latter appeared to be in poor condition from disease the previous winter (about one-third of the fish had died in a 3-month period).

In 1964, commercial fishermen in Wisconsin and Michigan caught 1725 Salmo Hatchery fish and 593 National Hatchery fish — a ratio of 2.9:1 in favor of the fish reared in Wisconsin. The addition of partial returns from 1965, however, indicated the ratio will be about 2:1.

Planting National Hatchery fish in Wisconsin resulted in only usual returns for fish of the size released (Table V). Returns of the Salmo Hatchery

TABLE V. Qualitative rating of success, size of fish at release, and station of rearing for various plantings of lake trout in U.S. waters of Lake Superior. (Plantings arranged in rank order of success within rating categories.)

Rating	Number of fish per pound	Hatchery[a]	Location of release	Year released
Excellent	17	National	Whitefish Point	1961
"	20	Salmo	Apostle Islands	1960
"	18	Salmo	Apostle Islands	1955
"	2	National	Marquette	1959
"	5	National	Marquette	1959
"	20	National	Munising	1960
"	27	Salmo	Apostle Islands	1961
Very good	18	Salmo	Apostle Islands	1956
"	7	National	Keweenaw Bay	1959
"	21	National	Whitefish Bay	1958
Good	23	Salmo	Apostle Islands	1958
"	22	Salmo	Apostle Islands	1959
"	26	National	Keweenaw Bay	1961
Fair	23	National	Marquette	1955
"	33	National	Pequaming	1960
"	22	National	Apostle Islands	1961
Poor	35	National	Marquette	1958
"	41	Salmo	Apostle Islands	1952–53
"	43	National	Marquette	1953–54
Very poor	80	Salmo	Apostle Islands	1954
"	37	National	Munising, Grand Marais	1958
"	58	Harrietta	Whitefish Bay	1962

[a]National – National Fish Hatchery in Michigan; Salmo – Salmo Hatchery (state) in Wisconsin; Harrietta – Harrietta Hatchery (state) in Michigan.

fish were, however, exceptional and were higher than for two other groups of large Wisconsin fish. Heavy culling of the weaker fish by disease or possibly the all-meat diet fed during convalescence may have contributed to the high survival. Buettner (1961) reported that, of the two year-classes he studied, the 1954 year-class which suffered heavy mortality from "blue sac" and "white spot" diseases subsequently survived at least 1.5 times as well as the 1955 year-class which suffered only normal hatchery mortality.

Although the returns from the 1961 releases in Wisconsin suggested that fish reared at the Salmo Hatchery were hardier than those reared at the National Hatchery, the early results of the 1962 Wisconsin releases indicated only that size at release affects survival. Large (8.9 per lb) fish reared at the National Fish Hatchery and released in Wisconsin in early fall yielded percentage returns equal to those for large (18 per lb) fish reared at the Salmo Hatchery and released in late spring (partial 1965 returns totaled 0.22% for each group). Both groups of large fish showed a great advantage (4:1 ratio) over smaller (22.5 per lb) National Hatchery fish released in the same area in the spring.

Regular surveillance of several Wisconsin releases with both experimental and commercial gear has shown that success or failure of a planting is determined before early fall of the year of release (2 to 3 months after planting). The physiological "hardiness" of hatchery-reared fish at release could be an important factor in ability of the fish to adapt rapidly to lake conditions. Unfortunately, no measures of "hardiness" other than size at release have been determined.

No other factors were obviously related to success of stocking. Abundance of native lake trout, either large or juvenile, did not appear to be related to stocking success in 1952–61; highly successful releases were made in years of both high and low abundance of native fish. Data collected by experimental gear in Wisconsin since 1961, however, suggest that survival from plantings has declined as the number of fish released each year and abundance of both juvenile and legal-sized lake trout have increased (abundance of legal fish increased roughly threefold during 1961–65). Although American smelt (*Osmerus mordax*) eat the same food and are commonly at the same depths as juvenile lake trout, abundance of smelt appeared to be unrelated to returns from stocking. Sea lamprey predation undoubtedly reduced the total return from many releases, but changes in abundance of sea lampreys showed no relation to the success of various releases. Mortality during the first few months after release appeared to be far more important than sea lamprey predation in determination of the returns from a release. Lamprey predation appeared to be confined mainly to fish as large as or larger than the average size taken in commercial nets.

Although selective breeding of lake trout for rapid growth to increase the average size at release might appear to offer a possibility of improving success of stocking, the late maturity of lake trout (5 to 7 years in hatcheries) makes early results unlikely. The only method that clearly offers early improvement of stocking success is to rear lake trout to optimum size for release (17 to 20 fish per lb). Any alteration of rearing practices (including heavy culling of small, weak fish) that would advance attainment of this size should result in consistently higher success of planting lake trout.

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Comparative Electropherograms of the Family Catostomidae[1]

By H. Tsuyuki, E. Roberts, and R. H. Kerr

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver, B.C.

AND

J. F. Uthe and L. W. Clarke

Fisheries Research Board of Canada
Freshwater Research Institute, Winnipeg, Man.

ABSTRACT

The muscle myogens of *Catostomus catostomus* exhibit polymorphism. Specimens from Great Slave Lake and Babine Lake were homozygous to pattern type A, whereas those from Lake Superior possessed patterns homozygous to A and B as well as the heterozygote AB. Those of *C. commersoni*, *Moxostoma macrolepidotum*, *M. anisurum*, and *M. erythrurum* were relatively uniform, showing only odd variants. The two specimens of *Ictiobus bubalus* examined displayed different patterns. Blood hemoglobin patterns of *M. macrolepidotum* existed in five, *M. anisurum* in two, and *C. commersoni* in three polymorphic forms. The number of specimens of *I. bubalus*, *M. melanops*, and *Carpiodes cyprinus* examined was insufficient to assess the existence of polymorphism. Muscle myogen electropherograms of the eight species investigated reflect overall family resemblances, distinctive generic groupings as well as phylogenetic relationships which compare favorably with those in existence. Intergradations might occur upon a more extensive coverage of this family and which might somewhat obscure this overall picture are discussed.

INTRODUCTION

In recent years increasing attention is being drawn towards finding biochemical characteristics of fishes to supplement existing biological knowledge. One aspect, the electrophoretic properties of blood and tissue proteins, has found application in problems involving identification of difficult species, as well as in uncovering intraspecies polymorphisms (Moller and Naevdal, 1966; Sick, 1961; Tsuyuki et al., 1965, 1966; Uthe et al., 1966). The latter information promises to be of value in population analyses (Frydenberg et al., 1965). The electropherograms of one of the tissue protein groups, the muscle myogens, appear to reflect phylogenetic relationship at the generic and family levels (Tsuyuki and Roberts, 1966; Tsuyuki et al., 1965) in species examined so far.

The electrophoretic properties of the proteins of approximately 100 marine and freshwater species have been described. To our knowledge, similar characteristics have not been described for the catostomid family, which

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consist of some 14 living genera of about 80 species (Miler, 1958). The family is divided into three subfamilies (Hubbs, 1930). Both Cycleptinae and Ictiobinae consist of 1 tribe and 2 genera and the Catostomidae of 3 tribes and 10 genera. The morphology of the four highly modified anterior vertebrae, the Weberian apparatus, connecting the swim bladder with the inner ear (Nelson, 1948) has been largely instrumental in this subdivision.

In this communication, zone electrophoretic characteristics of muscle and blood proteins and their polymorphic forms of eight species of catostomids representing five genera are described. It is also pointed out that these properties are specific characteristics and their usefulness complement biometric data in the description of a species as well as provide further criteria for population analyses. The catostomid family provides another example of phylogenetic relationships expressed in muscle myogen patterns.

METHODS

The muscle extracts and the blood proteins were prepared for starch gel zone electrophoresis as described previously (Tsuyuki et al., 1962, 1965). Plasma proteins were separated by polyacrylamide disc electrophoresis (Tsuyuki and Roberts, 1965).

RESULTS

Catostomus catostomus

The muscle myogens of *C. catostomus* display polymorphism (Fig. 1). The area of the electropherograms in which the variation occurred is represented diagramatically and involves combinations of three electrophoretically distinct proteins. These are designated by homozygous patterns A and B and by heterozygous pattern AB. All Great Slave Lake specimens examined, of which six are shown, are indisputably of pattern A whereas those from Lake Superior possess both the homozygous A and B patterns as well as the heterozygous AB pattern. These patterns are not sex-linked, as A type of Great Slave Lake and the A and B type of Lake Superior appear in either sex. Further sampling of 17 specimens of this species from Babine Lake in British Columbia revealed the existence of pattern A only.

In the plasma protein patterns of *C. catostomus* from Lake Superior and Great Slave Lake little systematic uniformity was observed either within or between the two lake systems (Fig. 2A and 2B). Of the Lake Superior specimens, no obvious correlation was apparent between the plasma proteins and the different myogen patterns. The uniformity of myogen electropherograms observed in the Great Slave Lake samples was not reflected in their total plasma protein patterns.

No individual variation was found in the multiple hemoglobin patterns of this species from Lake Superior. Electropherograms shown in Fig. 2C are typical for this species and do not reflect either sexual dimorphism or correlation with the different myogen pattern types. Great Slave Lake hemo-

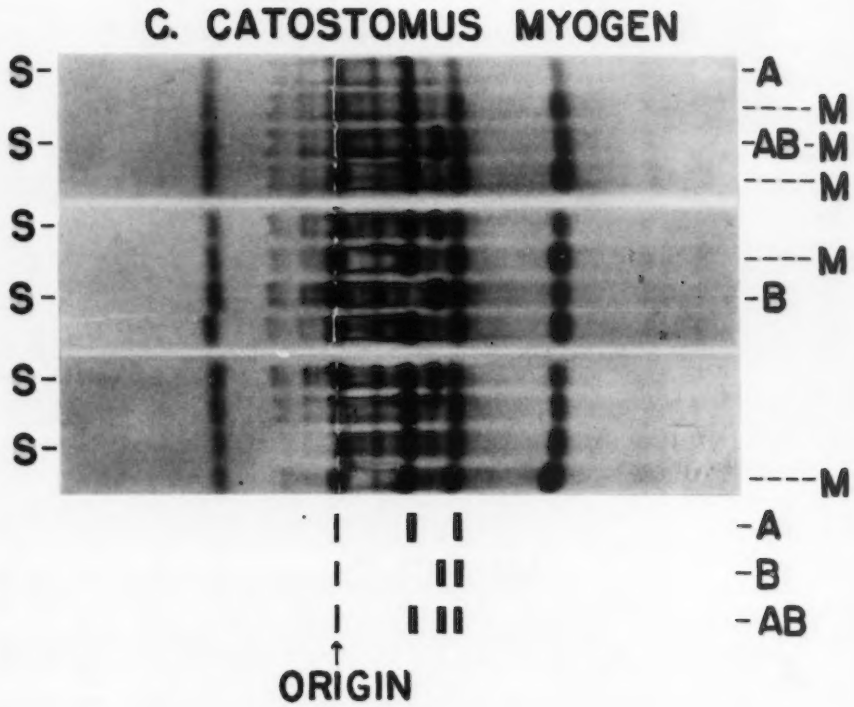

FIG. 1. Electropherograms of the muscle myogens of *C. catostomus*. Lake Superior samples are labelled S, the Great Slave Lake ones are unlabelled. Male specimens are labelled M and females are unlabelled. A, B, and AB designate the area of the electropherogram where polymorphism was found. In this and subsequent figures the anode is to the right and the cathode to the left.

Tsuyuki et al. — J. Fish. Res. Bd. Canada

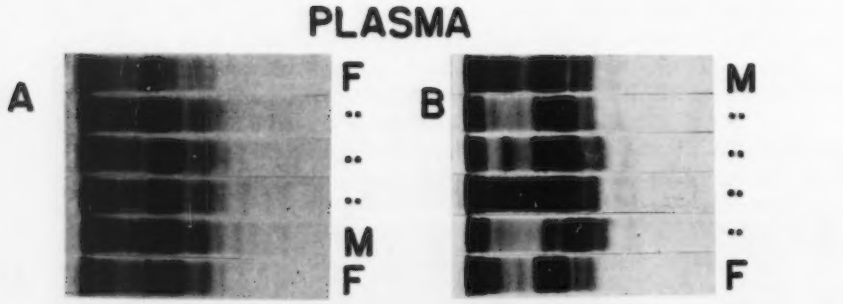

FIG. 2. Polyacryamide disc electropherograms of the plasma proteins of *C. catostomus* from Lake Superior (A), Great Slave Lake (B), and starch gel electropherograms of multiple hemoglobins of the same species from Lake Superior (C). F designates female and M males.

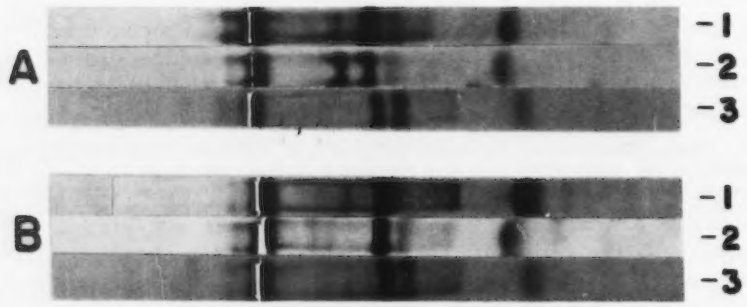

FIG. 3. Starch gel electropherograms of the muscle myogens of *M. macrolepidotum* (A) and *M. anisurum* (B). Numbers 1, 2, and 3 designate the variant patterns in each species.

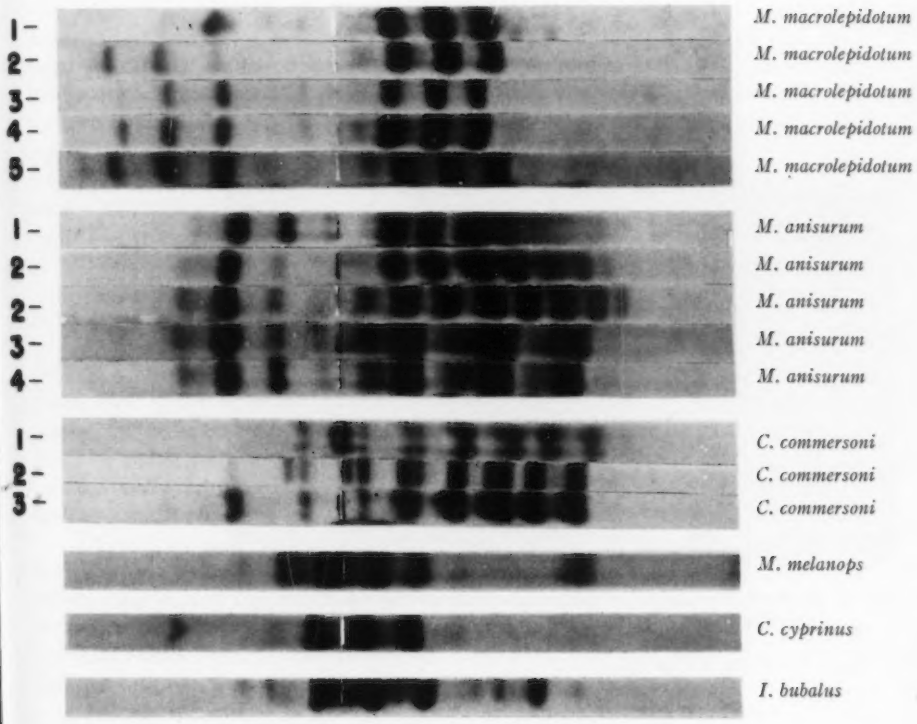

FIG. 4. Starch gel electropherograms of the multiple hemoglobins of some members of the catostomid family. The numerals represent polymorphic patterns obtained for the species shown.

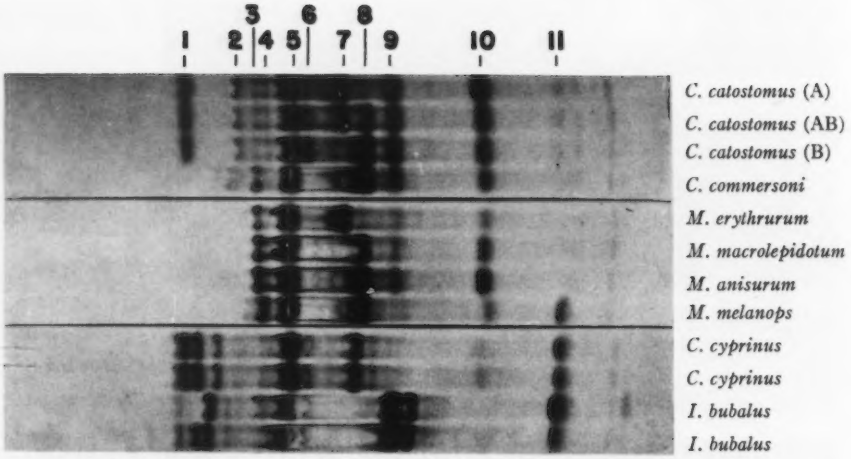

FIG. 5. Comparative starch gel electropherograms of the muscle myogens of eight species of catostomids. A, AB, and B denote the polymorphic patterns of *C. catostomus*.

globins were deteriorated so badly due to excessive time involved in transportation to Vancouver that their electropherograms were of little use. Seventeen specimens from Babine Lake showed the same patterns.

Moxostoma macrolepidotum, *M. anisurum*, *M. erythrurum*, and *C. commersoni*

The muscle myogen electropherograms of *M. macrolepidotum* are shown in Fig. 3A. Of 49 specimens, 47 showed pattern type 1, and one of each pattern types 2 and 3. Type 1 pattern is assumed here to be typical for this species. All but two of the specimens were females which, being larger than males, were selectively captured by the pound nets employed. Of 21 specimens of *M. anisurum* examined, 19 showed muscle myogen pattern 1, and one of each pattern types 2 and 3 (Fig. 3B). Types 2 and 3 differ from each other scarcely more than in relative concentrations of some of the protein zones. The virtual identity of the myogen patterns of *M. anisurum* and *M. macrolepidotum* is shown more clearly in a simultaneously run electropherogram in Fig. 5. Since the 25 specimens, representing approximately equal numbers of both sexes, of *C. commersoni* and the 8 specimens of *M. erythrurum* did not show any variant pattern, those shown in Fig. 5 are believed to be representative for these species.

The multiple hemoglobins of six species of the Catostomidae are shown in Fig. 4. Five general types of patterns were found in *M. macrolepidotum*: three type 1, five type 2, six type 3, eight type 4, and two type 5. These variations all occur on the cathode side of the electropherogram. It might be well to point out here that the two species, *M. macrolepidotum* and *M. anisurum*, indistinguishable by their muscle myogen patterns are clearly distinguishable by their hemoglobin patterns.

Of the hemoglobins of *M. anisurum* examined, one type 1, two type 2, and six each of types 3 and 4 were recognizable. These patterns clearly can be segregated into two broad groups consisting of types 1, 2 and 3, 4 by virtue of major differences on the anode side. Variations within these two groups are essentially one of differences in concentrations and may not warrant further subgroupings.

All 27 specimens of *C. commersoni* examined fitted into three pattern types and consisted of seven type 1, 17 type 2, and three type 3, based on different combinations of three hemoglobin zones in the anode side. Multiple hemoglobin patterns from a single specimen of *I. bubalus* and representative patterns from three of *M. melanops* and from five of *C. cyprinus* are also shown in Fig. 4.

DISCUSSION

Electrophoretic characteristics of specific proteins provide further information to the general pool of knowledge in describing fish species. Frequently it is possible to uncover intraspecies genetic information which are not readily apparent through meristic and morphometric analyses and which

may find application in fishery biology in problems involving identification of population. Polymorphic forms of hemoglobins have been used in gene frequency studies (Frydenberg et al., 1965) of the Atlantic cod, *Gadus morhua*, along the coast of Norway. Other protein groups as serum transferrins have also been shown to exist in polymorphic forms in carp, *Cyprinus carpio* (Creyssel et al., 1964), and in some gadoid fishes (Moller and Naevdal, 1966; Moller, 1965). Organ proteins, tissue, and serum esterases (Nyman, 1965) and enolases (Tsuyuki and Wold, 1964) are also possible sources of useful information.

The muscle myogens represent another group of proteins exhibiting polymorphisms of value in population studies. The muscle myogens of *C. catostomus* along with *Stizostedion vitreum* (Uthe et al., 1966) and a marine species, *Anoplopoma fimbria* (Tsuyuki et al., 1965) provide examples of intraspecies genetic variations. In Great Slave Lake and Babine Lake area of British Columbia, *C. catostomus* variants homozygous only to the A pattern exist, whereas those variants homozygous to A and B patterns as well as the heterozygotes existed in the Great Lake system. Similar geographic isolation of the homozygous pattern was observed in *S. vitreum*. As we have observed frequently, with existing methods at least, protein polymorphisms in fish species are not necessarily demonstrable on a wide scale nor are they confined to any single tissue. The choice of protein groups to analyze is largely a matter of trial and error as usually no guidelines are available. The hemoglobins of *C. catostomus* examined, for example, were unexpectedly uniform. On the other hand, although the muscle myogens of *M. macrolepidotum*, *M. anisurum*, *M. erythrurum*, and *C. commersoni* were relatively uniform except for the odd deviation in patterns, hemoglobin polymorphisms were widespread and promise to be of some value in population analyses.

Closely related fishes appear to possess similar enzymic systems involved in the biosyntheses of a host of muscle proteins of similar electrophoretic mobilities (Tsuyuki and Roberts, 1966). Comparative electropherograms of muscle myogens of some members of the catostomid family also reveal this same empirical relationship (Fig. 5). *Catostomus catostomus* and *C. commersoni* share common zones 2, 3, 4, 5, 8, 9, and 10. Notably absent are zones 1 and 6. No other member of this family examined bears so close a similarity. The three species of *Moxostoma* share in common zones 3, 4, 5, 9, and 10 intergenerically with *Catostomus*. The intensity of zone 3 is somewhat similar to *C. commersoni*, whereas zone 9 is greatly reduced in concentration compared to the genus *Catostomus*. *Moxostoma erythrurum* alone possesses zone 7, the polymorphic protein of *C. catostomus* which is absent in *C. commersoni*. *Moxostoma macrolepidotum* and *M. anisurum* have virtually identical myogen patterns but different hemoglobins. *Minytrema melanops* shares zones 5, 8, and 10 of the genus *Moxostoma*. Zone 11, common to *Carpiodes* and *Ictiobus*, makes its appearance in *M. melanops* and zone 10 diminishes in intensity. *Carpiodes cyprinus* shares zone 5 and 10 with *Catostomus*, *Moxostoma*, and *Minytrema*, though the concentration of the latter zone is greatly diminished. Zone 11 is also common to *C. cyprinus*, *M. melanops*, and *I. bubalus*. On the

cathode side *C. cyprinus* displays general similarities with the polymorphic proteins of *I. bubalus*.

From the comparative myogen electropherograms in this study, there emerges a general pattern of relationship within the Catostomidae which in a broad sense follows that proposed through other approaches (Miller, 1958). It is realized, however, that only 8 of some 80 species and 5 of 14 living genera are represented in this investigation. Nevertheless, the species examined fall into readily recognizable generic groups which in Fig. 5 are arranged in the order of resemblance to *C. catostomus*. Whether generic groupings remain sufficiently distinct when the Catostomidae is considered in its entirety cannot be answered until an exhaustive study is carried out. The appearance of intergrade patterns may well make generic groups less distinct.

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The Biology of an Ampeliscid Amphipod Crustacean Sibling Species Pair[1,2]

By Eric L. Mills[3]

Department of Biology, Queen's University, Kingston, Ontario
and
Marine Biological Laboratory, Woods Hole, Massachusetts

ABSTRACT

The amphipod sibling species *Ampelisca vadorum* and *A. abdita* have an overwintering generation and a summer generation. Breeding begins above 8 C. In *A. abdita*, breeding is probably correlated with the full moon. Curves of growth in length are linear, except where growth slows in winter. Length–weight curves are exponential, and *A. vadorum* is slightly heavier than *A. abdita* at any given length. The slope of the length–weight curves is the same in both species. Growth in weight is not isometric; weight increases less rapidly than does length. In some allometric features the two species stop growth at different points on the same line, but other morphological features develop out of phase due to genetic differences in patterns of development. The two species differ in a number of biological features including egg size, metabolic contribution in egg production, relative gill area, and probably egg number. Reported "intersexes" are actually subadult males. The sex ratio in both species is always 1:1. Gonad growth and somatic growth have different temperature limits.

Tubes, tube building, and feeding are described. Three major functional groups of ampeliscids show marked morphological adaptations to feeding type and sediment. Free swimming occurs at times of breeding, ensuring fertilization of eggs and dispersal of young to new and favourable environments.

Ampelisca vadorum occurs on course sand, *A. abdita* on fine sand to mud. Range, temperature, and depth preferences of these and other North American *Ampelisca* species are outlined. The fauna associated with *A. vadorum* and *A. abdita* is described. The unique microtopography of *Ampelisca* areas affects the nature of the association. *Ampelisca abdita* is successful in a crowded environment because it grows fast and breeds early. Ecological, life history, and taxonomic evidence all indicate that *A. vadorum* and *A. abdita* are sibling species.

INTRODUCTION

The amphipod crustacean sibling species *Ampelisca vadorum* Mills, and *A. abdita* Mills were mistaken for the European species *A. spinipes* Boeck until recent years (Mills, 1963a, 1964a). These two species are now known to be very widespread in shallow marine waters of eastern North America from Maine to at least Louisiana, and are related to a group of amphipod species found in the West African-European region (Mills, 1965).

In this paper, I outline the general biology of these species, consider their ecological differences, and attempt to outline the selective forces and adaptations responsible for their origin and maintenance.

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[2]Contribution No. 1613 from Woods Hole Oceanographic Institution.
[3]Present address: Institute of Oceanography, Dalhousie University, Halifax, Nova Scotia.

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MATERIAL AND METHODS

Ampelisca abdita was studied on the intertidal flats of Barnstable Harbor, Massachusetts, at a locality used in a survey by Sanders et al. (1962), 41°43′ 12″N, 70°20′W. My data on *A. vadorum* comes from animals collected by Mrs S. W. Richards of the Bingham Oceanographic Laboratory, Yale University, and by myself. The collection locality was one studied by Sanders (1956) in his survey of the benthic communities of Long Island Sound-Station 1, 41°11′6″N, 73°08′48″W, depth 10 to 12 m. Sorting and measuring techniques are discussed in detail elsewhere (Mills, 1963b).

GENERAL LIFE HISTORY

Both species are quite similar in their life history, and in general the life history resembles that described for *Corophium volutator* Pallas by Watkin (1941). An overwintering generation begins to breed about mid-April, with an early production of adult males. Breeding activity is linked with water temperature, and begins at about 8 C. In Barnstable Harbor in 1962, *A. abdita* began to breed at 8.0 C on April 14. Water temperatures throughout the preceding winter never rose above 3.5 C and were close to 1 C from December to mid-March. The exact time at which breeding began in Long Island is not certain, but large numbers of breeding adults were present on May 24, 1962, when bottom temperatures of about 10 C could be expected (Riley, 1956, and unpublished data). If 8 C is the governing temperature, breeding could be expected by early May, a little later than for *A. abdita* at Barnstable Harbor.

A temperature in the range 8–10 C must indeed be important in initiating breeding in the overwintering generation; winter collections from several localities to the south of Cape Hatteras (including Beaufort, N.C., May River, S.C., Charlotte Harbor, Fla., and Nine-mile Bayou, St. Bernard Parish, La.) contain ovigerous females of one or both species. The collections were taken in various years from January to March, long before the months at which breeding begins in New England. Parr (1933) summarizes information showing that shallow water temperatures in winter from Cape Hatteras southward remain at or above 10 C, whereas north of Cape Hatteras temperatures drop well below that level. From this information it is clear that breeding may be maintained throughout the winter on the sorthern Atlantic coast, whereas breeding stops in New England and eastern Canada where winter temperatures go much lower than 10 C.

By early May ovigerous females become abundant and the number of adult males declines. A few adults of the overwintering generation persist until late June. Young of the overwintering generation (referred to as the "summer generation") became a noticeable part of the population by mid-May 1962, and by June 18, 1962 these animals had reached breeding size. This generation reached a peak of breeding about the end of the second week in July in both 1961 and 1962, although ovigerous females continued to be

present in small numbers until early October. Young produced by such a summer generation then overwinter, growing slowly until breeding occurs in late April of the following year.

The presence of summer generation ovigerous females from mid-June to September means that overwintering animals vary greatly in size (range 2.0–7.3 mm in November 1961 for *A. abdita* and 2.4–11.5 mm for *A. vadorum*). Breeding in the following spring is spread over a relatively long period as the smaller animals attain adult size.

This general outline of life history of both species is based primarily on the extensive collections of *A. abdita* from Barnstable Harbor, but a very similar picture is revealed by the less extensive collections from Long Island Sound—a winter generation lasting 8 months, followed by a summer generation lasting 4 months. There is no evidence that adults of either generation survive beyond breeding in either species.

Figures 1 and 2 illustrate the data on which the life history description was based. Modes of the size frequency distributions have been separated mostly by inspection, and in difficult cases by using the probability paper method of Harding (1949) and Cassie (1954). I have assumed that each size-group has an approximately bell-shaped distribution and that my somewhat arbitrary lines do not introduce a great error by cutting through the areas of intersection of overlapping curves. A short summary for both species is found in Table I. Variation in size within each generation is expressed by the statistic V, as defined by Simpson et al. (1960). High variability (V higher than 5 to 6) results from the long breeding period of each generation.

The data for *A. abdita* suggest that 1961 summer generation animals were noticeably smaller than those of the following year. Reasons for this will be considered later.

Figure 2 illustrates an interesting phenomenon in the life history of *A. abdita*. The histograms for October 5 and November 4, 1961 show a marked bimodality of the overwintering generation. This can be followed throughout the winter with the help of the probability paper method, though by spring most of the distinctive bimodality has disappeared, perhaps masked by variability in growth rates of various members of the population. The time difference between the modes of these two components of the winter generation (designated W_1 and W_2) is about 29 days, as determined from the growth curve for the species (Fig. 3). This corresponds exactly with the period between two successive full moons, and suggests that breeding takes place at greatest intensity at times of full moon. Evidence will be presented later that the bulk of summer breeding takes place at the time of spring tides and full moon. Summer generation animals show the same bimodality, especially obvious in the histograms for June 25 and July 14, 1962. The length difference here corresponds to about 22 days, indicating that the breeding cycle, if dependent on moon or tides, is not strictly correlated with highest spring tides or complete full moon. The length-frequency histograms for *A. abdita* have

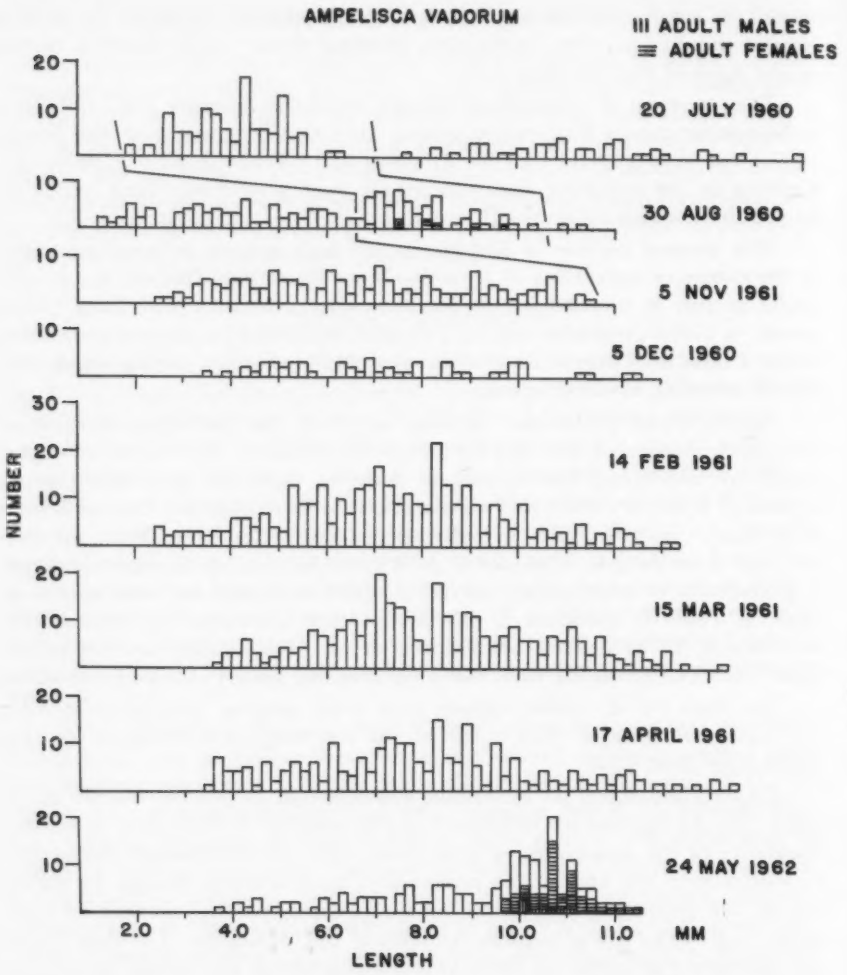

FIG. 1. Length-frequency diagrams for *Ampelisca vadorum*.

been analyzed in terms of these two components of each generation (S_1 and S_2; W_1 and W_2). Numerical data are summarized in Table II.

No solid evidence for bimodality can be obtained from the data on *A. vadorum*. This is not surprising, since these animals are entirely subtidal and the species sometimes occurs as deep as 70 m. Thus it might be expected that breeding behavior could be less easily correlated with tidal or lunar periodicity, features most easily perceived by intertidal populations. It would be

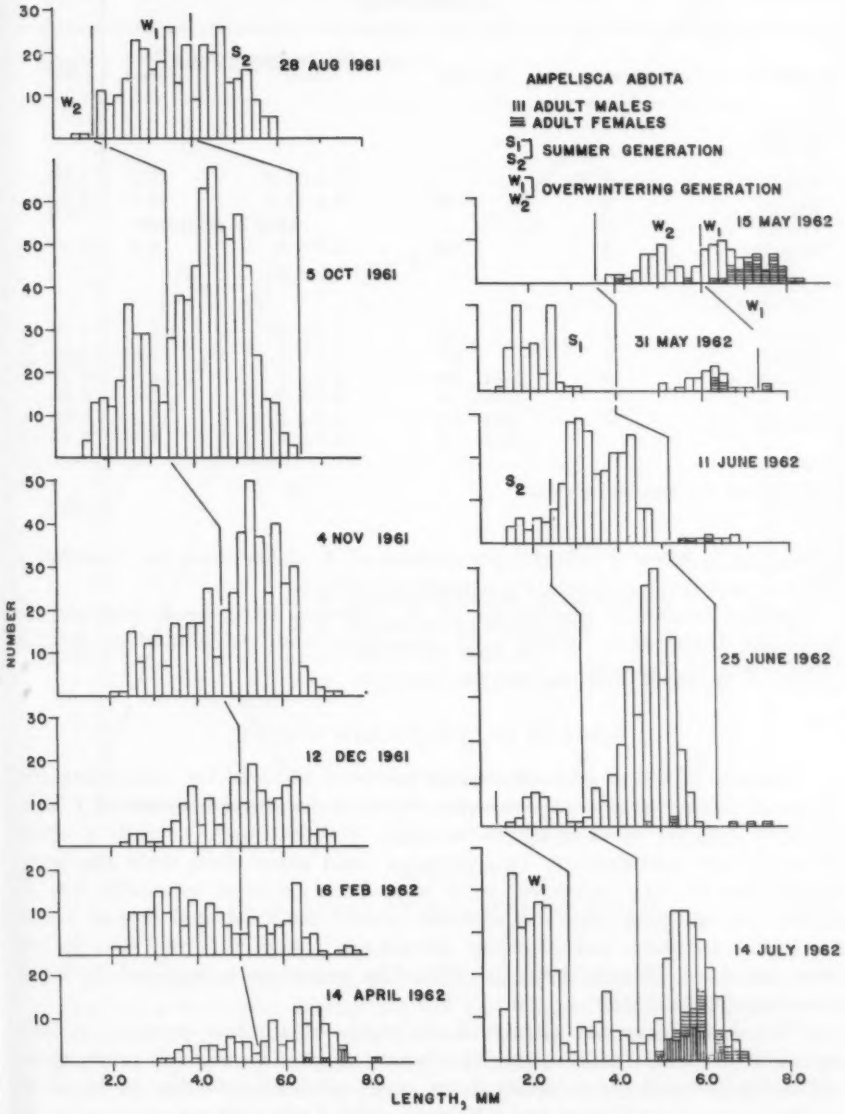

FIG. 2. Length-frequency diagrams for *Ampelisca abdita*.

TABLE I. Size and size variation in the sibling species *Ampelisca vadorum* Mills and *A. abdita* Mills.

Generation	Sex	Number	Size range (*mm*)	Mean (*mm*)	V[a]
Ampelisca vadorum:					
Winter	♂	7	10.0–11.0	10.3	4.97
Winter	♀	62	9.6–12.5	10.7	5.87
Summer	♂	None in collections			
Summer	♀	10	6.9–9.6	8.1	10.19
Ampelisca abdita:					
Winter	♂	19	5.6–8.0	6.53	9.76
Winter	♀	101	5.3–8.1	7.05	7.99
Summer	♂	1961: 257	4.0–6.3	4.93	6.81
		1962: 36	4.7–5.9	5.41	6.73
Summer	♀	1961: 219	4.6–6.8	5.44	7.99
		1962: 41	4.7–6.8	5.88	7.43

[a]Coefficient of variability, see text.

interesting to know if subtidal populations of *A. abdita* show the bimodality which occurs in the intertidal Barnstable populations.

Length-frequency histograms of *A. vadorum* have been analyzed as unimodal distributions within each generation and the numerical data is summarized in Table II.

GROWTH IN LENGTH AND WEIGHT

Growth in length of both species has been followed by calculating the means of the size-groups shown in Fig. 1 and 2. In using this method I have assumed that the series of length-frequency distributions is actually a series of length–age distributions. This seems a valid assumption since the same populations in very restricted areas have been sampled, especially for *A. abdita*. The resulting curves of absolute growth are illustrated in Fig. 3 and 4. From these data, instantaneous percentage growth rate per day, k, has been calculated (Brody, 1945, p. 508). The procedure is outlined in detail elsewhere (Mills, 1963b).

The most noticeable feature of the curves of absolute growth for both species is their essential linearity. This is most marked for summer generations, where the animals grow rapidly from newly released juveniles to breeding adults in a period of from 40 to 80 days, with little evidence of decrease in absolute growth rate as maturity is approached. Kinne (1954b) records a somewhat similar growth curve for *Gammarus duebeni* Lilljeborg. These curves differ considerably from the classical sigmoid growth curves usually assumed typical, especially in work on fish.

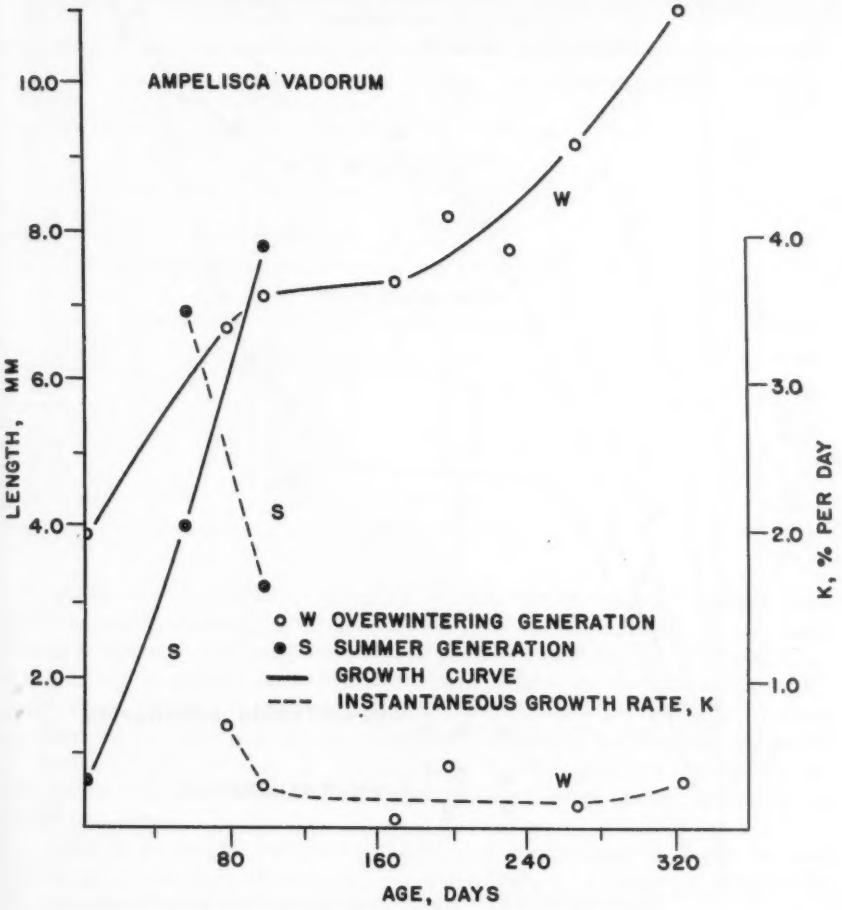

FIG. 3. Growth curves of *Ampelisca vadorum*.

The curves for the winter generation differ in showing a marked decline in absolute growth rate during late fall and winter. This is due solely to low water temperatures during this period. Growth resumes in almost linear fashion in the spring as water temperatures increase. Water temperatures are plotted with length values and values for k in Tables II and III (data for Long Island Sound from Riley, 1956), illustrating the marked decrease in growth with falling water temperatures. Taylor (1962) points out that inflection points in linear growth curves may be a reflection of conditions in the external environment, particularly temperature. Reduced availability of food in winter may be partly responsible for this decline in growth rate, but

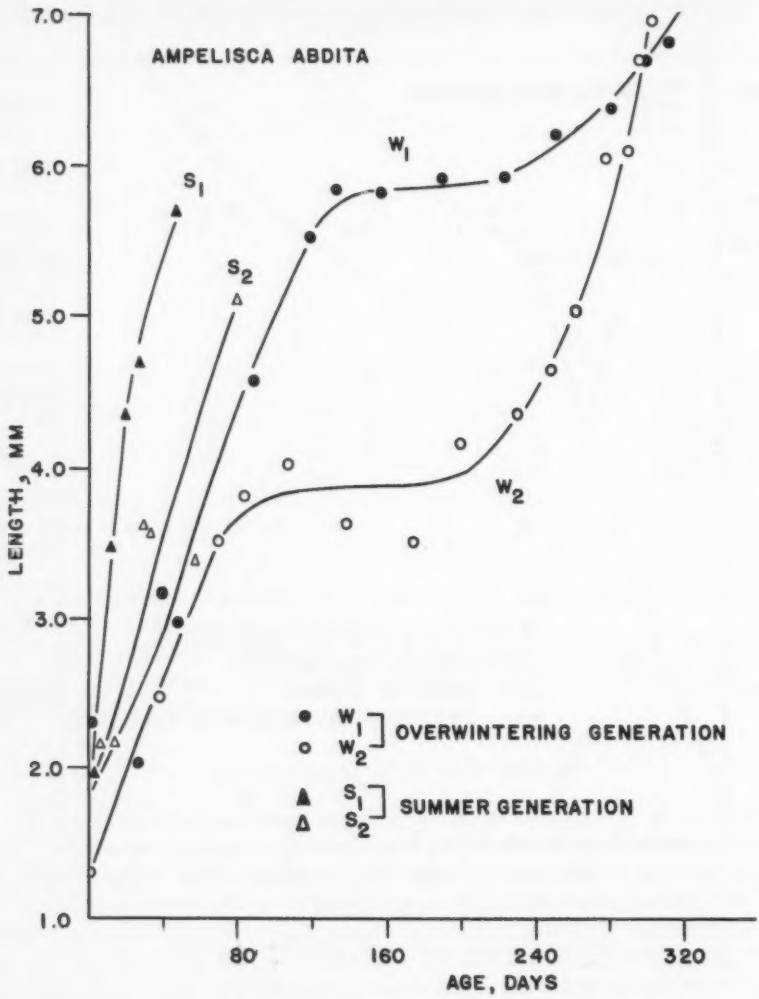

FIG. 4. Growth curves of *Ampelisca abdita*. For an explanation of the two curves for each generation, see text.

limited observations at Barnstatle Harbor suggest that the detrital food of these animals is quite abundant even at times of lowest winter temperatures.

The values of k listed in Tables II and III are quite consistent in both species, indicating a sharp decrease in percentage instantaneous growth rate per day from the early high values for summer generation animals. Values of k for winter generation animals decline a little less abruptly than for the

TABLE II. Range in size and growth in length of *Ampelisca vadorum* (k = instantaneous growth rate).

Date	Range (*mm*)	Mean (*mm*)	Water temperature (*C*)	k, % per day
	Summer generation (S)			
July 20, 1960	1.8– 6.2	4.02	18	3.47
Aug. 30, 1960	6.6–10.2	7.84	22	1.63
	Winter generation (W)			
July 20, 1960	7.6–15.8	11.09	18	0.326
Aug. 30, 1960 (a)	10.8–11.2 (a)	11.0	–	–
(b)	1.2– 6.4 (b)	3.89	22	1.358
Nov. 15, 1961	2.5–11.4	6.71	10	0.708
Dec. 5, 1960	3.4–12.5	7.12	9	0.295
Feb. 14, 1961	2.4–13.2	7.32	2	0.038
Mar. 15, 1961	3.6–14.2	8.27	4	0.421
Apr. 17, 1961	3.5–15.0	7.82	6	–
May 24, 1962	3.7–12.5	9.22	11	0.155

summer generation initially, indicating the late summer and fall growth, then reach a nearly constant level just above zero throughout the winter, with a final increase in the last burst of growth to breeding size in the April–May period. Erratic values for k of the summer generation S_2 component could result from patchy distribution of juveniles, giving incorrect mean values for the sizes in these populations. Recorded values of k for W_2 became erratic as winter progressed due to the difficulty of distinguishing this group as variability in growth began to mask the difference between the central values of the two groups.

Growth in weight for the two species is summarized in Table IV and Fig. 5. It is clear from these data that individuals of *A. vadorum* weigh more than similarly sized individuals of *A. abdita*.

The Y-intercepts of the lines in Fig. 5 can be shown to be different, using a suitable *t*-test, reflecting the observation that *A. vadorum* was significantly heavier than *A. abdita* at the same length. The slopes of the two lines are not significantly different, and a value of B (slope) = 3 would indicate isometric increase of weight with length (Ricker, 1958). The values obtained for *A. vadorum* and *A. abdita*, being significantly less than three, show that weight increases slightly less rapidly in growth than does length, and indicate that a change in form occurs with length. This change in form is shown in the allometric growth of some parts in the two species (Mills, 1964a), and is not a radical change as in many crustaceans, but a gradual lengthening and slimming of body parts, including antennae, head, peraeopods, and uropods.

TABLE III. Range in size and growth in length in *Ampelisca abdita* (k = instantaneous growth rate). Parentheses indicate unreliable values of k and estimated means.

Date	No./m²	Range (*mm*)	Mean (*mm*)	Water temperature (*C*)	k, % per day
Summer generation (S)					
May 31, 1962	4,000	1.3–3.0	1.98	18	–
June 11, 1962	11,050	2.4–4.7	3.49	23	5.153
June 18, 1962	34,850	3.2–5.7	4.35	–	3.147
June 25, 1962	66,400	3.3–5.8	4.69	–	1.075
July 14, 1962	45,000	4.4–6.8	5.57	25	0.905
b. Component 2 (S_2)					
June 11, 1962	1,200	1.4–2.3	1.87	23	–
June 18, 1962	11,150	1.2–3.1	2.15	–	1.993
June 25, 1962	7,000	1.4–3.0	2.16	–	0.066
July 14, 1962	11,800	2.9–4.3	3.57	25	2.645
Aug. 5, 1961	49,600	2.8–4.1	3.39 (4.15)	–	(0.684)
Aug. 28, 1961	27,800	4.0–5.9	5.12	25	0.913
Winter generation (W)					
a. Component 1 (W_1)					
July 10, 1961	1,400	1.6–2.7	2.30	–	–
Aug. 5, 1961	51,600	1.2–2.7	2.04	–	–
Aug. 28, 1961	36,400	1.6–3.9	2.99	–	1.662
Oct. 5, 1961	109,200	3.4–6.3	4.57	–	1.116
Nov. 4, 1961	60,800	4.6–7.3	5.53	–	0.636
Nov. 18, 1961	32,400	5.0–7.3	5.85	–	0.402
Dec. 12, 1961	22,400	5.0–7.1	5.83	3.5	0
Jan. 13, 1962	20,200	5.0–7.3	5.94	1	0.058
Feb. 16, 1962	12,400	5.0–7.6	5.94	1	0
Mar. 14, 1962	16,800	5.4–7.5	6.22	6	0.177
Apr. 14, 1962	4,050	5.4–7.5	6.41	8	0.097
May 2, 1962	4,000	5.6–8.0	6.74	9	0.279
May 15, 1962	3,500	6.0–8.2	6.86	14.5	0.136
May 31, 1962	100	–	7.4	18	0.474
b. Component 2 (W_2)					
Aug. 28, 1961	400	1.2–1.5	1.30	25.0	–
Oct. 5, 1961	37,000	1.4–3.3	2.48	–	1.699
Nov. 4, 1961	31,400	2.0–4.5	3.52	–	1.167
Nov. 18, 1961	20,600	2.3–4.9	3.82	–	0.584
Dec. 12, 1961	17,800	2.2–4.9	4.01 (3.84)	3.5	0.202
Jan. 13, 1962	22,800	2.2–4.9	3.64 (3.84)	1.0	(0)
Feb. 16, 1962	27,200	2.1–4.9	3.52 (3.85)	1.0	(0.008)
Mar. 14, 1962	22,800	2.6–5.3	4.17	6.0	(0.307)
Apr. 14, 1962	1,950	3.0–5.3	4.36	8.0	0.144
May 2, 1962	1,850	3.0–5.5	4.65	9.0	0.358
May 15, 1962	2,600	3.8–5.9	5.05	14.5	0.635
May 31, 1962	1,350	5.0–7.1	6.07	18.0	1.149
June 11, 1962	450	5.5–6.7	6.12	23.0	0.075
June 18, 1962	1,850	5.8–8.1	6.76	–	1.421
June 25, 1962	600	6.6–7.4	7.0	–	0.498

TABLE IV. Length–weight data (averages) for *Ampelisca vadorum* and *A. abdita*.

Length (*mm*)	Weight (*mg*)	
	A. abdita	*A. vadorum*
1.2	0.010	
1.5	0.020	
1.7	0.030	
1.8	0.033	
1.9	0.030	
2.3	0.095	
2.4	0.080	
2.5	0.080	
2.8	0.185	
3.0	0.200	
3.1	0.165	
3.2	0.190	
3.3	0.160	
3.4	0.163	
3.5	0.213	
3.7	0.240	
3.8	0.220	
3.9	0.285	
4.0	0.317	
4.1	0.305	
4.2	0.295	
4.3	0.454	
4.4	0.490	
4.5	0.533	0.880
4.6	0.560	
4.7	0.518	
4.8	0.507	
4.9	0.623	
5.0	0.538	0.884
5.1	0.790	
5.2	0.728	
5.3	0.605	
5.5	0.767	0.944
5.6	0.640	
5.7	0.835	
5.8	0.747	
5.9	0.720	
6.0	0.885	1.265
6.1	0.772	
6.2	0.860	
6.3	0.735	
6.4	1.190	
6.5	0.880	1.581
7.0	–	1.676
7.1	1.360	
7.3	1.323	
7.5		2.083
8.0		2.455
8.5		2.934
9.0		3.318
9.5		3.904
10.0		4.880
10.5		5.368
11.0		6.198
11.5		6.832
12.0		7.320
12.5		7.808
13.0		8.540
13.5		9.272
14.0		9.760
14.5		10.980

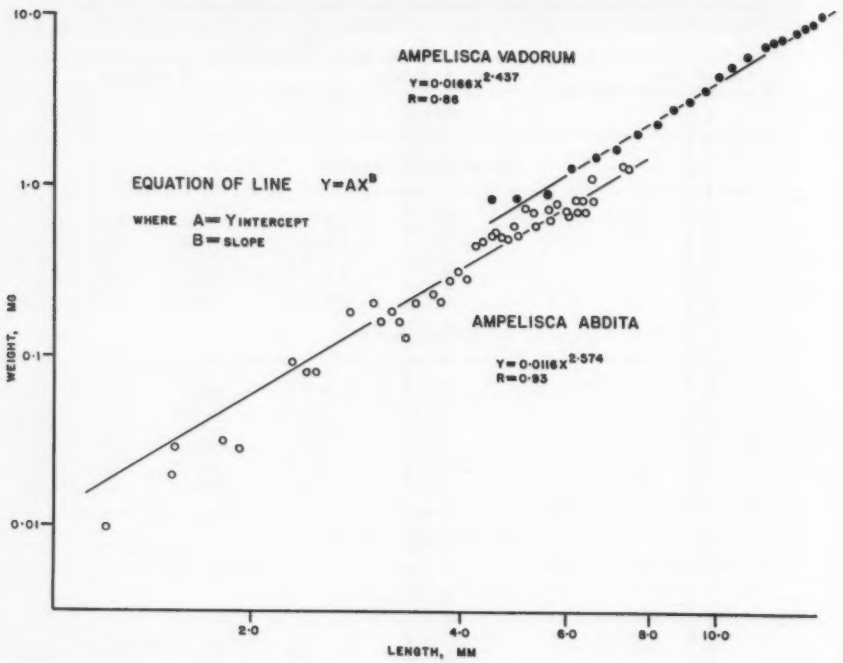

FIG. 5. The relationship between length and weight in *Ampelisca vadorum* and *A. abdita*.

OTHER STATISTICS OF THE SPECIES

A number of other measurements of mature females are summarized in Table V. Where 95% confidence limits overlap, the significance of difference in means has been tested by a *t*-test.

Egg number is statistically inseparable in both species at the 5% level of significance. The large range of values in both species is not wholly due to intrinsic individual differences in egg number, but is partly due to loss of eggs from some females upon initial preservation and when preserved animals are handled for measuring or removed from a vial for examination. The loss is probably greatest in *A. vadorum*, since the eggs are only slightly larger than in *A. abdita*, whereas the fringing setae of the oostegites, which retain the eggs in the marsupium, are more widely spaced and more flexible. Thus the statistically inseparable egg numbers could be significantly different if the figures were computed from animals which had not lost eggs. Egg size is noticeably smaller in the smaller species *A. abdita*, and falls within a rather small range of values in both species.

Absolute gill area is considerably larger in *A. vadorum* than in *A. abdita*, as might be expected from their size differences. The measurement of gill

TABLE V. Biological statistics of *Ampelisca vadorum* and *A. abdita* ovigerous females.

		Means with 95% confidence limits (N = 10)	
		A. vadorum	*A. abdita*
1.	Egg number	32.1±7.94	26±4.06[a]
2.	Egg size, *mm*	0.556±0.021	0.427±0.015
3.	Body volume/egg volume	165.94±16.81	49.09±7.24
4.	Gill area, mm^2	14.19±1.52	4.33±0.36
5.	Gill area/body volume, mm^2/mm^3	0.955±0.289	2.177±0.351
6.	Gill area/body weight, mm^2/mg	2.292±0.216	3.547±0.416[b]

[a]Not different statistically, using Student's *t* test.
[b]Statistically different, using Student's *t* test.

area was complicated by the pleating of the gills in both species. This pleating is a simple, wave-like folding of the body of each gill, effectively increasing the surface area over that of a flat plate. The pleating is best developed in *A. vadorum* and is present but often not well developed in *A. abdita*. It was not possible to measure pleating effectively due to its complex geometry, so the gill areas shown in Table V represent the area of a flat plate. Adding the surface area contributed by pleating to the areas given here would increase both slightly, and probably also slightly increase the ratios based on gill area. Since the comparison of the two species is the important point of discussion, this inaccuracy in the absolute values and of the ratios is not of great importance to the conclusions to be derived later.

The statistics numbered 3, 5, and 6 in Table V have been calculated in an attempt to arrive at some conclusions about metabolism, life history, and ecology of the animals which may be compared with observed features of life history and ecology of the two species. These conclusions will be put forward in a later section.

SEX RATIO AND REPRODUCTION

A supposed abnormality in the sex ratio of the sibling species considered here was one of the original reasons for the initiation of this study. Reid (1951) found difficulty in working with members of the *Ampelisca diadema-spinipes-tenuicornis* group collected off West Africa due to the presence of "intersexes" — apparently males with female characters. Many of the specimens he examined had copulatory hooks on the peraeopod 4 and 5 sterna (typical of males), plus oostegites (typical of adult and subadult females). Sanders (1956) mentions Reid's view on the "intersex" phenomenon and states that females usually outnumbered males by at least 15 to 1 in Long Island Sound. Of 100 specimens of *Ampelisca* A (*A. vadorum*) examined, Sanders found that 50%

had normal female characters, whereas 50% were "gynomorphic males" or "intersexes." Barnard (1960) comments that for California sublittoral ampeliscids "sexually mature males are exceedingly rare, generally in the proportion of 1 to 50 or 1 to 100."

The first suggestion as to the status of these "intersexes" and of adult males came from the collection of *A. abdita* made at Barnstable Harbor on July 10, 1961. At that time large numbers of fully adult sexually mature males were present, and the ratio of males (adult and subadult) to females was 1:1. Juveniles, which could not be sexed, were not included in the calculations. I have assumed that the juveniles also have a 1:1 sex ratio. After this collection the number of adult males dropped precipitously. With the growth of the new generation it became clear that the female-like "intersexes" made up about half the population and were actually subadult males. My data for *A. vadorum* (Table VI) show that this group is consistently 50% of the

TABLE VI. Sex ratio data for *Ampelisca vadorum*.

Date	♂ ♂	Juvenile ♂ ♂	♀ ♀	Juveniles (<6 *mm*)
Nov. 15, 1961	–	43	49	62
Dec. 5, 1960	–	17	19	20
Feb. 14, 1961	–	96	101	144
Mar. 15, 1961	–	130	134	45
Apr. 17, 1961	–	93	91	52[a]
May 24, 1962	7	70	101	–[a]

[a]Male/female ratio statistically inseparable from 1:1 (juveniles not included).
NOTE: Sex-ratio tested statistically only for April 17, 1961 and May 24, 1962, when most juveniles have come into adult categories (6 mm and over).

post-juvenile population. Sexually mature males of both species appear late, probably at a definitive last moult which almost amounts to a metamorphosis, as described for *Pontoporeia affinis* Lindström by Segerstrale (1950). The same modifications, such as slimming of the body, increase in pleon muscle size, more slender antennae, and broadening and increased setation of the third uropods, occur in both *A. vadorum* and *A. abdita* and are associated with improved swimming ability and adaptation for a short adult life, free-swimming in the water column.

Evidence from morphology reinforces the other evidence that the "intersexes" are actually subadult males. In both *A. vadorum* and *A. abdita* as breeding times approach, the new cuticle of the adult male may sometimes be seen under the cuticle of the subadult male. The morphological features easiest to observe in these subadults are the incipient increased carination of the first urosome segment and the great increase in the segmentation of the flagellum of antenna 2.

Segerstrale's (1950) comments on *Pontoporeia affinis*, in which he likens the last moult of the male to a metamorphosis, have been mentioned. The male of *P. affinis* is sufficiently different from the female and juvenile males that it was known for a time as *P. weltneri*, and similar confusion has occurred more than once in the taxonomy of the genus *Ampelisca* (Mills, 1963a).

There is no evidence for true intersexuality in either *A. vadorum* or *A. abdita*. However, both these species lack penial (copulatory) hooks, and this makes identification of intersexes more difficult than in species where the hooks occur. Collections of *A. spinipes* from Europe in my possession show a high incidence of intersexuality. Large specimens bearing both bristleless (that is, subadult female) oostegites and the copulatory hooks of males are relatively common. These animals may conceivably grow to a larger size than normal adults due to delayed breeding activities and hence may be more easily collected in sampling operations, particularly trawls, than the smaller normally sexed animals. Barnard (1960) has suggested a similar reason for the presence of large, aberrant "gerontic" individuals in collections of southern California ampeliscids. Thus, intersexuality apparently does occur in some species of the genus *Ampelisca*, and it has also been reported in *Gammarus* species (Sexton and Huxley, 1921; Sexton, 1924), although it has proved to be of minor importance in the biology of *A. vadorum* and *A. abdita*.

An examination of the sex ratio data for *A. vadorum* (Table VI) shows a consistent 1:1 relationship when all adults are considered. However, in *A. abdita* (Table VII) the picture is not so clear. At breeding times, when the numbers of adults are high and most juveniles are long enough (>4 mm) to be considered in the adult categories, the sex ratio is indeed 1:1. At other times considerable variation is evident in my collections. There are two possible reasons for this. First, it is conceivable that juvenile males grow somewhat more slowly than juvenile females. Thus a higher proportion of males would remain in the unsexed "juvenile" category, causing an apparent displacement of the sex ratio in post-juveniles towards female. Second, it may be that there is a behavioral difference between juvenile males and females, causing clumping of like-sexed animals. The displacement of the sex ratio would reflect only the discontinuous distribution of the sexes. There is no certain evidence for either suggestion, but examination of Table VII shows that the number of subadult males is consistently below that of females throughout the winter and into April, suggesting that the hypothesis of slower growth, not that of discontinuous distribution, is the correct one. The fact that adult males are consistently smaller than adult females is further evidence for this hypothesis (see Table I). A suggestion of the same phenomenon in *A. vadorum* may be seen in Table VI.

It is quite clear that the sex ratio of both species is 1:1 at times of maximum breeding. This is in accordance with the ideas of Fisher (1930, p. 143) who emphasized that selection will tend to balance the sex ratio at 1:1 except under unusual circumstances.

TABLE VII. Sex ratio data for *Ampelisca abdita*.

Date	♂ ♂	Juvenile ♂ ♂	♀ ♀	Juveniles (<4 *mm*)
July 10, 1961	263	83	310	228*
July 30, 1961	8	90	185	972
Aug. 5, 1961	8	19	69	256
Aug. 28, 1961	–	55	95	193
Oct. 5, 1961	1	214	207	319
Nov. 4, 1961	–	167	192	109
Nov. 18, 1961	–	99	111	57
Dec. 12, 1961	–	66	92	45
Jan. 13, 1962	–	53	86	78
Feb. 16, 1962	–	51	46	100
Mar. 14, 1962	–	65	99	38
Apr. 14, 1962	7	29	77	10[b]
May 2, 1962	2	36	75	5
May 15, 1962	–	50	72	–[a]
May 31, 1962	–	17	11	80
June 11, 1962	–	50	21	183
June 18, 1962	15	255	263	433
June 25, 1962	2	136	166	64
July 14, 1962	34	79	122	267

[a]Male/female ratio statistically not different (juveniles not included).
[b]Male/female ratio statistically different (juveniles not included).
NOTE: Sex ratio has been tested statistically only when most juveniles have come into adult categories (4 *mm* and over).

The situation in these *Ampelisca* species is not in accordance with Kinne's (1961b) finding that the normal sex ratio in *Gammarus duebeni* Lilljeborg is about 1 ♂:3 ♀ ♀, varying considerably with environment. Hart (1930) found a similar sex ratio in *Corophium volutator* Pallas. Kinne suggests that this displaced sex ratio is directly adaptive, since *G. duebeni* females, which have a relatively long life cycle, can lay eggs several times. Males may copulate with several females in the course of life, and thus the species can have greatest reproductive success by reducing the relative number of males to the minimum necessary to ensure fertilization of all females and by increasing the relative number of females, thus greatly increasing egg production over the value possible with a 1:1 sex ratio. In *G. duebeni* sex ratio may be displaced towards males under suboptimal conditions and Kinne feels this displacement may have great adaptive significance, since if males are more resistant than females under these suboptimal conditions they may maintain the species in an area by occupying space there until more favorable conditions allow the relative number of females to rise. In addition, the presence of relatively large numbers of males could interfere with the success of competing species of the same genus, since it has been observed that males of one species of *Gammarus* will mate with females of closely related species, but that the cross

is always sterile. Thus by mating with females of competing species, *G. duebeni* males might reduce the egg production of those species.

Kinne's argument, which I feel is quite tentative, is that to ensure complete adaptation to environmental conditions, the sex ratio of amphipods must be environmentably modifiable. He suggests that sex determination is temperature-dependent and capable of adaptation. In *G. duebeni*, females produced only males if the temperature during a "sensitive phase" before oviposition was below 5 C, produced mixed broods if the temperature was between 5 and 6 C, and produced only females if the temperature was above 6 C. In addition, he found that all reproductive processes, including egg number, moulting frequency, and precopulation time, were temperature dependent.

Whatever the temperature relations of the reproductive processes of these *Ampelisca* species may be, the 1:1 sex ratio usually observed in *A. vadorum* and *A. abdita* is as directly adaptive as the 1:3 ratio observed in *Gammarus duebeni*. Since *Ampelisca* females produce only one brood before death, it is essential that each reproductive female be found immediately by a reproductive male. This life history signifiance of the 1:1 sex ratio will be further explored in the following section.

The mechanism of sex determination in amphipods has been a subject of speculation for some years. Palmer (1926), in his examination of the chromosome of *Gammarus chevreuxi* Sexton, suggested that a Y-chromosome was present in males, although later authors' findings threw some doubt on his observations. Orian and Callan (1957) observed no certain heterochromosomes in various members of the family Gammaridae but they did observe a variation in chromosome number in some species. This variation was apparently due to the occasional formation of small supernumerary chromosomes. Niiyama (1959), in an extensive survey of crustacean karyotypes, suggested that an XY or XO genetic sex determination mechanism is present in all Crustacea. In amphipods this heterogamety might not always be obvious, since amphipod chromosomes are notoriously many and small.

Evidence for the physiology of sex determination in amphipods has been summarized by Charniaux-Cotton (1960, 1962). In brief, the mechanism is as follows. The juvenile amphipod gonad differentiates spontaneously into an ovary as growth progresses. In females, hormones from the ovary initiate the growth of the oviduct and oostegites and such secondary sexual characters as the oostegite bristles of full adults. In genetically determined males the growth of an androgenic gland occurs in the region of the vas deferens. Its hormone causes regression of any ovarian cells in the gonad and initiates spermatogenesis, as well as controlling development of male secondary sexual characters. Transplantation of the androgenic gland into females before the terminal moults will masculinize them; removal of the glands from males before the terminal moults will feminize them.

The phenomenon of intersexuality, mentioned previously in *Ampelisca spinipes*, may be explained by some of Charniaux-Cotton's work (1962). In Talitridae intersexuality may occur by at least two processes. First, the

action of the androgenic glands in genetically determined males may be delayed, allowing the ovary and some other female sexual characters to develop. Second, males may be feminized even when the androgenic gland functions early by some genetic modification of the androgenic hormone. It would be interesting if this genetic modification were correlated with the frequent chromosome anomalies observed by Orian and Callan (1957). Charniaux-Cotton observes that intersexes are not favored in nature, since intersex males cannot remain in precopula with normal females. Since it is apparently maladaptive, it seems possible that intersexuality may be correlated with some cytogenetic instability. This instability might also help to account for the easy displacement of sex ratio observed by Kinne (1961b).

The actual process of pairing and fertilization is not known in *Ampelisca*, but is known in other amphipods (Holmes, 1903; Crozier and Snyder, 1923; Hynes, 1955; Orian and Callan, 1957). Mature males, in their wanderings, come in accidental contact with other amphipods of the same species and grasp them automatically. Other males struggle and are released, but females nearing the time of egg production are passive and are carried about by the male (Holmes, 1903). This stage is known as precopula. At the end of the precopula period the female molts, and while the cuticle is still soft begins to lay eggs. The male now shifts its body transversely to that of the female and bends its abdomen into the brood chamber, releasing sperm into it from the openings of the vasa deferentia at the base of the fifth peraeopods. After fertilization, male and female may continue to be linked together for a considerable period. In *A. abdita*, and probably in *A. vadorum*, sexually mature animals and some juveniles leave their tubes and swim freely at time of spring tides and full moon (as discussed previously). Fertilization must occur at this time. Males disappear very quickly from the population, and females return to the substratum, dying only after the young animals have left the brood chamber. Specificity in the mating process is due primarily to the reactions of the female when grasped by a male, and to the size of the pair attempting to mate, according to Crozier and Snyder (1923) who found that even within a single species, "*Gammarus locusta*" (probably *G. oceanicus*), large males tended to mate with large females and small males with small females. Other combinations were infrequent, due to the mechanical difficulties of precopula with different-sized partners. These observations suggest a physical mechanism for the maintenance of size distinction in sympatric populations of *A. vadorum* and *A. abdita*. Mating between the two groups would be reduced by the inability of different-sized animals to stay coupled, even if genetic differences did not cause intersterility.

Both males and females of *A. vadorum* and *A. abdita* bear flattened, sensilla-like hairs on the first flagellum segment of antenna 1. These hairs are best developed in males, indicating that they have a function in reproduction, perhaps the location of the female, although Holmes (1903) suggested that in other species females were found only accidentally, by random movement. In animals such as ampeliscids, which are sedentary and bottom-dwelling

for most of their lives, random movements for location of members of the opposite sex could be less likely than a chemical sense which might operate at a particular stage of the life cycle, such as the brief period in the water column.

Data on egg number have been summarized previously (see Table V). The number falls within the values for various Gammaridae (Cheng, 1942). When comparison is made with Kinne's (1961a) values for *Gammarus zaddachi* Sexton, *A. vadorum* falls a little below the values observed for similarly sized animals of *G. zaddachi* (11–13 mm), and *A. abdita* is considerably above the values recorded for the smallest ovigerous females of *G. zaddachi* (8 mm). Retention of eggs by the female occurs in all amphipods and development is direct, with the juveniles leaving the brood pouch at a size of about 1.5 mm in both species and after about 2 weeks in the brood chamber in *A. abdita* (the time for *A. vadorum* is not known). This time is similar to that in *Corophium volutator* (Watkin, 1941) but seems quite short when compared with Kinne's (1960) data for *Gammarus salinus* Spooner and *G. duebeni*, even though Kinne's data is expressed as time between oviposition and "hatching." By "hatching" Kinne apparently means not the time at which the juvenile becomes free-living but the time during its period in the brood pouch when the embryo loses its egg-like form and emerges from the egg membranes.

Egg production occurs only once in both *A. vadorum* and *A. abdita*, with death of the mature animals occurring after this first reproduction. This is unlike the situation in *G. duebeni* (and most *Gammarus* species), where oviposition may occur six to seven times and length of life under moderate temperature conditions ranges from 275 to 810 days (Kinne, 1954b, 1959, 1961b) as compared to about 90 days in *A. vadorum* and *A. abdita*.

SIGNIFICANCE OF GROWTH, LIFE HISTORY, AND REPRODUCTION

The preceding sections have outlined what is known of the growth and life history of *A. vadorum* and *A. abdita*. It is now necessary to review these data in a more general way to investigate the adaptive significance of the biological facts. The importance of such an attempt has been stressed by Cole (1954) who outlined the meaning of several life history features in determining survival of animal populations.

The shape of the growth curves of both species is of considerable interest. The general nature of the curves has been discussed previously and the curves are illustrated in Fig. 3 and 4. The curves of absolute growth differ from the sigmoid curves usually mentioned in text books, and are essentially linear. In this respect they differ from the curves calculated for *Daphnia magna* by Anderson (1932), and come closest to those for other Amphipoda, such as *Pontoporeia affinis* (Segerstrale, 1950) and *Gammarus zaddachi* (Kinne, 1961a). However, curves for these two species show a flattening at the upper end which is definitely not present in the two species considered here. Also, the curves for *Ampelisca* show little evidence of the short early period of slow

absolute growth typical of *Daphnia magna* and characteristic of the sigmoid growth curve. Ricker (1958, p. 192), in an outline of the growth of fishes, states that "The changeover from increasing to decreasing length increments may be so slow and protracted as to make the age–length relationship effectively linear for almost the whole of the fishes life...." In the case of *A. vadorum* and *A. abdita*, growth is rapid and the linearity cannot be looked on as resulting from slow change from increasing to decreasing increments. Rather it seems that the ends of a more usual growth curve have been strongly modified, for adaptive reasons to be explored shortly.

No attempt has been made to calculate equations for the growth curves of Fig. 3 and 4, since the actual form of the curves rather than the formal mathematical description of curve shape is most useful here. The essentially empirical nature of growth curves was stressed by von Bertalanffy (1957), who nevertheless attempted to correlate metabolism and growth in various groups of animals in terms of primary metabolic parameters. Von Bertalanffy suggested that three primary metabolic types exist. (*1*) A type in which respiration (that is, metabolism) is proportional to the two-thirds power of weight. Metabolism is proportional to the animal's surface area. This type is characterized by a sigmoid curve of growth in weight and a reverse exponential curve of growth in length. Growth is limited when weight-proportional catabolism becomes equal to surface-proportional anabolism. (*2*) A type in which respiration is directly proportional to weight. Curves of growth in length and in weight are both exponential. No steady state occurs in growth, only a continual increase. (*3*) A type intermediate between the preceding two types in which metabolism is intermediate between surface and weight proportionality. Curves of growth in length and weight are both sigmoid.

The *Ampelisca* species appear to be intermediate between the second and third types, with growth in length being of a severely modified sigmoid form and growth in weight being exponential. It is possible that in these species cessation of growth, in the usual sense of a balance of anabolism and catabolism (von Bertalanffy, 1957), never has a chance to occur and that death of adults, which is of ecological advantage to juveniles in increasing living space and reducing competition for food, is linked with cessation of activity of the gonad after its production of eggs or sperm. It has apparently become important for these species to grow quickly to reproductive size, to breed, and then to die quickly.

Two factors apparently limit growth. The first factor is temperature, and its effect in slowing growth rates is clear in Fig. 3 and 4. In both species, growth may continue slowly through the winter to a considerable size, well beyond the size at breeding in the summer generation. Thus low temperatures have a greater retarding effect on gonad differentiation than on somatic growth. During the summer, a temperature limitation of gonad differentiation does not occur, and general somatic growth appears to be restricted by the rapid growth of the gonad to breeding condition. Thus gonad growth and differentiation is the second factor limiting body growth. It appears that the temperature limits

of gonad growth and somatic growth are quite different, with 8–10 C the required temperature for gonad development whereas considerable somatic growth can occur at least down to 3–5 C. In immature animals the gonad is visible through the cuticle (as an orange-yellow mass in females), occupying a large part of the dorsal area of the body cavity. It would be interesting to compare gonad weight or volume in these species with that in species which grow more slowly and produce several broods of young in the course of a long life history. Apparently no data exist for such a comparison, but it would be expected that the gonad of *Ampelisca* would be relatively larger and would play a larger role in metabolism than in longer-lived, slower-growing species.

Cole (1954) lists a number of means by which a species may increase its biotic potential, including increase in litter size, reduction of mortality to the end of reproductive life, increased frequency of reproduction, reproduction at an early age, and reduced importance of sterile members. A number of these factors are important in the life history of *A. abdita* and *A. vadorum*, increasing their chances of success in environments which are extremely crowded and in which biological competition must be extreme. The nature of this competition will be discussed in the concluding parts of this paper.

Increase in litter size appears to have played little role in the success of *A. vadorum* and *A. abdita*. The mean number of eggs for both species falls within the range noted in other species such as *Gammarus zaddachi* (Kinne, 1961a), *G. duebeni* (Kinne, 1959), *G. pulex* Linnaeus, *G. tirgrinus* Sexton, and *G. lacustris* Sars (Hynes, 1955). There is no doubt that interspecific differences in egg number exist, related to the life history and ecological situation of each species. The considerable difference in egg number between similarly sized females of *G. zaddachi* and *A. abdita* has already been noted. Table V suggests that egg number is similar in *A. vadorum* and *A. abdita*, but as previously noted, there is evidence for a slightly higher number in *A. vadorum*. This difference might be looked on as sufficient to balance an extra mortality of *A. vadorum* due to its slightly longer prereproductive period. Lack (1954), however, cautions against this naive view of the action of mortality and natural selection, pointing out that selection will only favor individuals producing the maximum number of possible offspring, whether this number is above or below the mortality level of the species. Although his conclusions which were derived for birds may not apply strictly here, egg number must be, nonetheless, the result of a more complex mechanism than response to species mortality. The probable factors involved will be discussed shortly.

Reduction of mortality to the end of life is not utilized as a means of increasing biotic potential in these species. An examination of the calculated numbers of *A. abdita* per square meter (Table III) shows an almost constant decrease throughout the winter, suggesting a continuous mortality and a survivorship curve of the concave-upwards type III or IV variety (Slobodkin, 1961, p. 35). A sudden mortality does occur at the end of life, however, when adults disappear leaving the environment relatively empty and with reduced intraspecific competition for the juveniles.

Increased frequency of reproduction is not a factor in ecological success in either species, since both breed only once per generation. Other means have been found for contending with the pressures for space and food. These means are primarily rapid growth and reproduction at an early age. The growth curves, particularly of the summer generation, show that both species grow exceedingly fast in almost linear fashion, with a time from egg to reproduction of 40 to 100 days under conditions of no temperature restriction, as compared with periods of 170 to 180 days in *Gammarus duebeni* (Kinne, 1954b), 90 to 120 days in *G. pulex*, over 300 days in *G. lacustris*, and 180 to 200 days in *G. tirgrinus* (Hynes, 1955). It is worth noting that *G. pulex*, with the shortest growth period of the species of that genus listed, has been replacing other species in some areas of the British Isles (Hynes, 1955). Cole (1954) points out that the actual number of offspring produced by a female is less important in the success of a species than the age schedule upon which young are produced. Early reproduction may often more than offset any disadvantage of small broods or single broods. Rapid population growth may often be best accomplished by crowding reproduction into the early life of the mother. This is just the case in both *A. vadorum* and *A. abdita*, where life histories and time of development are greatly shortened. The small *A. abdita* has carried these methods to the greatest extreme, a situation consistent with intense competition with other infaunal elements in its particular habitat (see discussion in a later section).

Reduction in importance of sterile members may be quite important in some *Ampelisca* species, though as previously mentioned there is little evidence for intersexuality in *A. vadorum* and *A. abdita*. Where intersexes do occur their appearance must be looked on as either due to a balanced polymorphism, and thus in some way adaptive, or as the result of cytological instability. In either case, their contribution to reproductive success is nil, and interference is reduced by the inability of intersexes to remain coupled with normal females (Charniaux-Cotton, 1962). It is of course possible that intersexes in some *Ampelisca* species have the same role as that suggested for males of *Gammarus duebeni* at times of suboptimal conditions (Kinne, 1961b), that is, they may serve to keep competitors from an area simply by occupying space in it. This must be balanced against the obvious disadvantage of having consuming but non-reproductive members of the population which contribute nothing to the next generation.

Some of the biological statistics in Table V must be regarded in the light of Brody's (1945, p. 580) statement that "Large and small animals are not similar geometrically, but rather tend to be physiologically, i.e. homeostatically similar. The organism changes geometrically so as to remain the same physiologically. The age curves of growth in different dimensions or in different components tend to be so adjusted as to give the animal mechanical stability and metabolic or physicochemical homeostasis."

It seems that the two species differ in several characteristics by being at different positions on a line of allometric growth, with *A. abdita* stopping its growth at a considerably smaller size than does *A. vadorum* (Mills, 1964a). This could be true of the gill area/body volume and gill area/body weight

measurements, although they have not been put to the test. If *A. abdita* adults were to increase in size, these ratios could be expected to approach those of adult *A. vadorum*. But they do not increase in size, and the result is that *A. abdita* adults have greater respiratory surface in relation to metabolically active weight or volume (see Table V). This evidence can be used to suggest either that adults of *A. abdita* have a higher metabolic rate than do those of *A. vadorum*, or that they occur in an environment in which oxygen is in shorter supply than is the case for *A. vadorum*. There is no certain evidence for either hypothesis, but the ecological evidence suggests that the relatively larger gill area of adults of *A. abdita* allows them to inhabit areas with lower oxygen tension, such as fine sand and mud sediments.

It was suggested earlier that egg number is slightly higher in *A. vadorum* than in *A. abdita*, and that this cannot merely represent a difference in mortality. Table V shows that the egg represents a much greater proportion of the body volume in *A. abdita* than in *A. vadorum*, and is thus a greater metabolic contribution in the former species. As stated previously, both species increase their fecundity by growing fast and breeding early. These three facts taken together outline the balance of advantages and disadvantages of size, time of growth, and metabolism of egg production in the two species. *Ampelisca abdita* is favored by breeding early at a small size, and the early breeding overcomes the disadvantage of producing slightly fewer eggs. Eggs probably cannot be reduced much in size without adversely affecting the development of the juvenile, or without reducing the size of the juvenile, which would decrease chances of survival in a crowded environment. Thus *A. abdita*, to ensure survival of its juveniles, must make a proportionately larger metabolic contribution to egg production than is necessary for *A. vadorum*. In *A. abdita*, selection should tend to increase egg number. This would involve reduction of egg size and so be disadvantageous. Rapid increase to breeding size overcomes this necessarily lower egg number.

Sex ratio has been discussed before, along with ideas on the ecological significance of the ratios in various species. In *A. abdita* and *A. vadorum*, the 1:1 sex ratio is an adaptation in the same sense as other life history phenomena. Since the growth curve is essentially linear, with females producing only one brood before death, males may be able to fertilize only one female, unlike the situation in *G. duebeni*. The 1:1 sex ratio assures fertilization of all females. Hynes (1955) finds a 1:1 sex ratio on a year-round basis in several freshwater *Gammarus* species, so the phenomenon observed by Kinne (1961b) cannot be universal, and his explanation of sex ratio displacement bears further examination.

Finally, the data on growth and metabolism suggest a mechanism for the evolution of a form like *A. abdita*. I assume that *A. vadorum* has been the ancestral form because it is now the more widespread and more eurythermal of the two species, and because it falls within the range of size of most related species in the diverse European-West African fauna. If, in isolated populations of *A. vadorum*, it became advantageous during times of environmental insta-

bility to have larger relative gill area as adults and increased fecundity associated with quick growth to breeding size, specific differentiation could occur. The zoogeographical evidence and a further discussion of the evolution of the two species has been summarized elsewhere (Mills, 1965).

TUBE-BUILDING AND FEEDING

Life in a tube is a dominant feature of the biology of both *A. vadorum* and *A. abdita*. The tubes of *A. abdita* are about 3½ cm long and 2–3 mm wide at the mouth end, flattened laterally, and composed of a grey, non-chitinous, parchment-like material and fine sand grains (Fig. 6). Tube mouths may be up to a centimeter above the sediment surface. Where densities are high, such as at Barnstable Harbor, the flat sides of tubes are oriented perpendicular to the current, probably allowing an increased surface for food catching (see remarks on feeding behavior to follow). This orientation also increases sediment deposition around the tubes, counteracting their tendency to wash out. The tube of *A. vadorum* has different proportions and may be about 2½ cm long and 5 mm

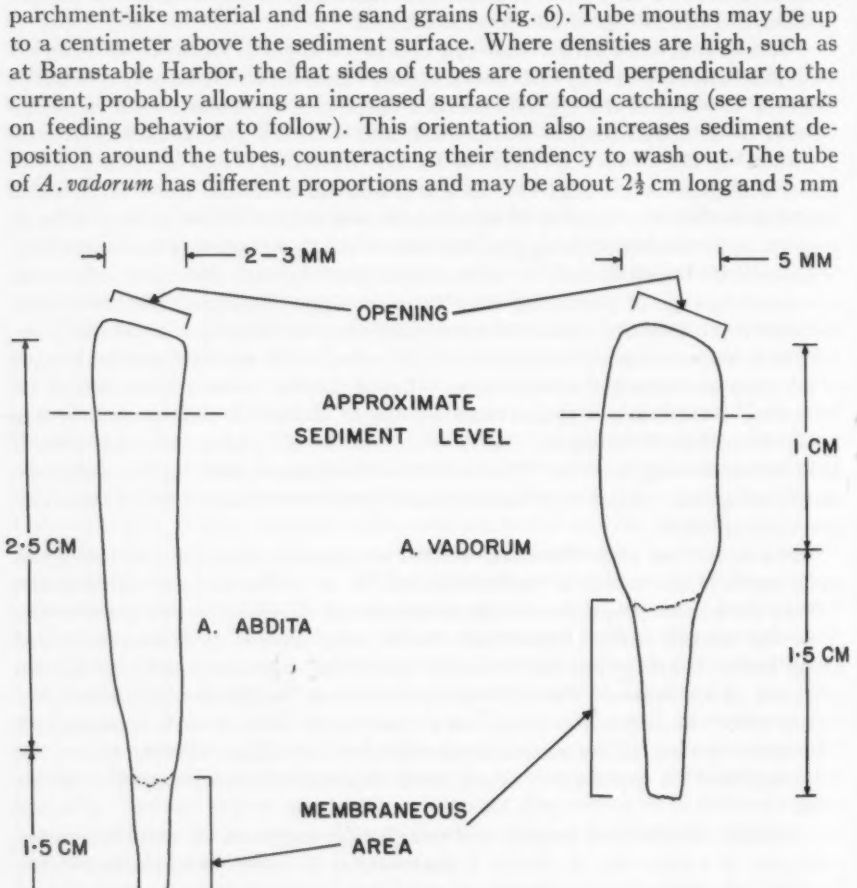

FIG. 6. The tubes of *Ampelisca vadorum* and *A. abdita*.

wide at the mouth (see Fig. 6). The difference in form must be due at least in part to the coarser and harder sediments in which this species lives, making construction of a long tube difficult.

The mechanism of tube-building is not well known, but my observations on *A. abdita* give some clues to the process. An animal placed on a fine sand substratum burrowed rapidly, dorsal surface uppermost, with powerful thrusting movements of the abdomen and with the aid of a strong, backwardly directed pleopod current sweeping sand posteriorly. During this burrowing the gnathopods and first two pairs of peraeopods manipulated sand grains and masses of detritus below the animal. At this stage the second antennae also gathered sand and detrital material toward the body. A loose accumulation of sand and debris was formed about the animal in 5 min. Tube-building began when the animal turned on its side or with ventral surface up and formed a frame of about three threads around the body, stretched between head and uropods. The threads were secreted by the massive glandular tissue in peraeopods 1 and 2 through ducts on the dactyls and were stretched out around the body by the movement of the urosome and uropods posteriorly. The gnathopods placed sand particles in this initial structure to give the first loose accumulation. Within this loose framework the animal then somersaulted 180° and began accumulation of sand particles lower in the tube. Small patches of sand particles bound up in glandular secretion were added to the framework here and there, building it up in patchwork fashion, leaving openings at the top and bottom. The top opening became the mouth of the finished tube, and the bottom opening was gradually attenuated as the tube was built downward (Fig. 6).

The final tube has a quite different aspect from the first accumulation of sand grains just described. Although the intermediate processes are not known, they seem to involve building up and down. Very fine particles are used finally on the outside of the tube. The tube lining is whitish and smooth, a pure glandular secretion lacking sand grains. The finished tube is somewhat elastic and the body of the animal forces the mouth apart for feeding. When the animal withdraws the tube mouth shuts automatically.

The motions of gnathopods, peraeopods, urosome, and pleopods in the tube-building process are very similar to those used in feeding (see later remarks), burrowing, and swimming. This is an interesting economy, allowing a few basic movements to be used in slightly different combinations to achieve a number of purposes.

The size and length of tubes is dependent on the size of the animals constructing them. Juveniles build tiny narrow tubes which must rapidly become too small, making construction of a new tube necessary, although there is no direct evidence for this activity. Larger animals must also occasionally build new tubes as growth makes the old ones too small. During the winter, activity is greatly slowed and the tubes become much less conspicuous as the sediment level rises around them. Some winter tubes have a transparent membranous area about 3 ml below the mouth that looks much like the membranous area at

the extreme base of the tube. This is apparently a region where building has been renewed to keep the tube above the surface of the substratum.

Enequist (1950) describes a similar process of tube-building in the European mud-bottom ampeliscid *Haploops tubicola* Lilljeborg. Free animals dig into the substratum with the dorsal surface uppermost. Superficial mud is moved by the gnathopods and the pleopod current, then burrowing occurs by the process just described for *A. abdita*. The animal then turns its ventral surface uppermost and begins to gather particles with the second antennae and gnathopods. The mouthparts work the particulate material which has been mixed with secretions from the peraeopods. This material is pulled out around the body by stretching with the uropods just as in *A. abdita*. A somersault then allows the animal to work downwards in the tube. Tube-building goes on at a rate of about 2 cm in 4 days. When the tube extends downwards 3 or 4 cm, the animal begins to work upwards until the tube extends 1 cm above the surface. Material for building is carried from the bottom of the tube to the top.

All ampeliscids feed in the tops of their tubes with the ventral surface turned upward. The body is held in place by the long finger-like dactyls of peraeopods 1 and 2 which loop over the sides of the tube, by the pressure of the reflexed peraeopods 3 and 4 against the sides of the tube well within it, and by the pressure of the spiny uropods against the posterior end of the mouth opening. The first and second antennae extend from the tube while the animal is in this position. The head projects from the tube while the animal is in the tube mouth, with its anteroventral aspect directed upwards. In this position the four simple eyes are uppermost and are able to detect movements anywhere around the tube. Antenna 1 is the last appendage to disappear when the animal retreats into the tube, and it may frequently be seen partially extended from the tube mouth even when it is closed. This evidence, as well as the presence of sensillae on the first article of the flagellum, suggests a chemical or tactile sensory function for antenna 1.

Feeding is apparently initiated by turbidity of water or currents around the tube. In the laboratory, light has no role in initiating and stopping feeding, and my preliminary laboratory experiments indicate that temperature has little effect on the nature of feeding behavior, although the rate of activity is greatly slowed below 10 C. Currents and turbidity are indications of the presence of suspended food in the surrounding water and are likely to be the primary cues for feeding activity.

Two general methods of feeding are common in *A. abdita* and *A. vadorum*. Occasionally the second antennae pick up sand grains from the sediment surface and cast them in to the midline of the body, where they may be held by the gnathopods and worked over with the mouth parts. Sand grains are then discarded by the gnathopods. In a variant of this feeding method an animal may tip forward and grasp sand grains with its mouth parts, work them over, and then cast them away with a quick movement of the mouthparts or gnathopods.

The second and most common method of feeding (Fig. 7) involves currents set up by the pleopods and second antennae. The metachronal beat of the

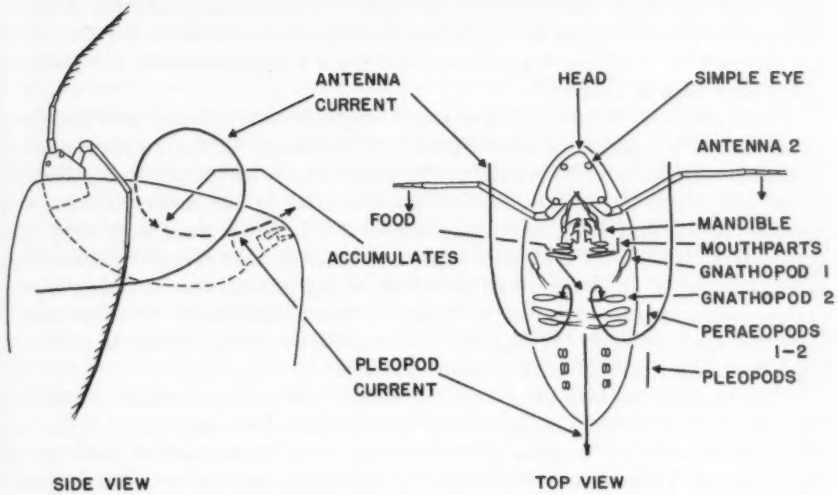

FIG. 7. Feeding position and currents in both *Ampelisca vadorum*. and *A. abdita* (diagrammatic).

pleopods (described in detail for *Haustorius arenarius* (Slabber) by Dennell, 1934) causes a strong current to move posteriorly along the midline of the animal between the coxal plates. This current passes water over the gills and aids the feeding process. The first antennae are held directly upwards, motionless, whereas the second antennae are whirled rapidly in synchrony beside the body in a "forwards-outwards" then "backwards-inwards" motion which may be as rapid as 116 beats per min. The effect of this movement is to set up currents which run posteriorly along the outside of the tube, then curve upward and turn downward towards the midline of the body (see Fig. 7). This current converges with the backward-directed pleopod current at the level of the second gnathopod and first two pairs of peraeopods. The latter in particular are heavily set with plumose setae on the basis and merus. Sand grains and detrital material accumulate on the filter screen formed across the midline by these setae. Large particles are rejected by the second antennae.

The first gnathopods are held somewhat to the side of the midline during the first parts of the food gathering process. Every few seconds, with the accumulation of food material on the midventral screen, the first gnathopods close towards the midline, scraping food material off the posterior limbs and passing it forward to the mouthparts. Maxillipeds and maxillae have a vibrating motion toward the midline at this time. Hart (1930) describes the lateral and medial movements of the maxillipeds in *Corophium volutator*. Food is scraped from the second gnathopods on the inward movement and passed to anterior mouthparts by the forward bending of medial spines on the outer plates. Such a process undoubtedly occurs in *A. abdita*. When food material reaches the heavily

sclerotized molars of the mandibles it is ground and passed into the foregut. The heavily ridged molars and well-developed incisor processes of the mandibles are adapted for scraping organic material from sand grains and for grinding particulate organic material.

This account of the feeding method suggests that detritus and the flora associated with sand grains are the main food materials. In the few specimens of *A. abdita* examined, food material in the anterior part of the gut was yellowish green and relatively unconsolidated, whereas that in the posterior part was greenish brown and hard and consolidated. The gut contents were very fine sand grains, tiny spherical objects (possibly bacteria), tiny pieces of filamentous algae, tetrads of algal cells, and numbers of diatom valves. Examination of the gut contents gives little clue to what is actually digested, but it seems likely from this examination that diatoms and unicellular algae as well as partially degraded plant material are the main food sources.

The feeding method of *Ampelisca* species has been described in various ways by a number of authors. Hunt (1925) referred to European forms as "suspension feeders." Sanders (1956) referred to *A. abdita* and *A. vadorum* as "filter-feeders." Enequist (1950) regarded European species as "detritus feeders," and his term best describes the situation in *A. abdita* and *A. vadorum*. Filter or suspension feeding does occur, but it is combined at times with swirling up of sand grains from the bottom, almost a form of deposit feeding.

Enequist's paper (1950) and my observations on the feeding method of *A. abdita* and *A. vadorum* indicate that a marked ecological partition occurs within the family Ampeliscidae. Enequist (1950) records the feeding method of *A. brevicornis* (Costa) and several other species and summarizes the differences between the two main feeding methods he observed. *Haploops* feeds more or less passively, with its head away from the current, using the equally long first and second antennae as a net to catch suspended material carried by currents or settling downward in the water, whereas *A. brevicornis* actually scrapes the bottom using the long second antennae, while the very short first antennae are inactive. In an intermediate third category are *A. abdita* and *A. vadorum*, which use the long second antennae to set up feeding currents to concentrate suspended or resuspended material, while the moderately long first antennae are inactive.

First antenna length, general body form, tube height, and morphology and sediment preferences seem to be correlated with the three main feeding methods just described. These methods may be more explicitly stated as:

(*1*) *Passive sifting* — These species beat second antennae only in still water and then beat only irregularly and independently. Usually sift detritus from water currents. Both pairs of antennae are long and setose. Tubes built high above sediment surface, too high to allow antennae to touch surface. Occur in areas of deposition, usually muddy regions. Includes *Haploops tubicola*, *H. setosa*, *Ampelisca amblyops*, *A. anomala*, and *A. aequicornis* according to Enequist (1950), and on the basis of morphology probably also *Byblis longicornis* Sars, *B. erythrops* Sars, and *B. crassicornis* Metzger. Urosome keel low, not massive, designed for use in soft sediments.

(*2*) *Whirling* — Usually beat the second antennae regularly and in synchrony to set up feeding currents. Antenna 1 held immobile vertically. May occasionally scrape bottom with second antennae and often whirl up detritus from sediment surface. Antenna 1 considerably shorter than antenna 2, but usually extending beyond its peduncle. Tubes extend moderately far above sediment surface, but low enough to allow second antennae to touch surface. Occur in areas of deposition, as well as on fine to coarse sands. Includes *Ampelisca spinipes* and *Byblis gaimardi* (Kröyer) according to Enequist (1950), *A. vadorum* and *A. abdita* according to my observations, and probably *A. odontoplax* Sars and *A. eschrichti* (Kröyer). Urosome keel or boss (a thickened dorsal ridge or blunt projection) well developed in some species, probably correlated with burrowing in coarse hard sediments.

(*3*) *Scraping*—These species scrape the bottom with second antennae, leaving circular marks on the sediment surface and moving masses of detritus and sand grains towards middle of body. First antennae usually very short, with few setae, held vertically upwards in water; only second antennae used in feeding, sometimes rest on bottom beside tube. Tube mouths to within 1 ml of sediment surface. Usually in fine sediments including fine sand and mud. Includes *Ampelisca brevicornis*, *A. macrocephala* (Kröyer), *A. tenuicornis* Lilljeborg, *A. diadema* (Costa), and *A. gibba* according to Enequist (1950), and probably also *A. typica*. Urosome keel moderately developed.

Several comments must be made about these three categories. They indicate a clear-cut differentiation, which is not entirely the case. Species in all three categories may utilize any one of the three methods of food gathering at some time or other, but generally specialize in only one. In addition, passive sifters and scrapers should generally be found on soft sediments, but *A. macrocephala* is an exception in being on coarse sand sediments, at least in North America, and *A. typica* is characteristic of very coarse sand and gravels in Europe, according to Crawford (1937). The urosome carina of *A. typica* is, however, well developed and probably an adaptation for life in coarse sediments. Crawford's notes (1937) on the distribution of several other species agree well with the arrangement above. *Ampelisca brevicornis* and *A. tenuicornis* are found on fine muddy sands as expected, and *A. spinipes* is on "mixed grounds." *Ampelisca diadema* occasionally departs from the expected fine sediments to go on "mixed grounds," but is often found with *A. tenuicornis* on finer sediments. *Ampelisca gibba*, although certainly a scraper, may build no tube according to Enequist's aquarium observations. The one specimen observed burrowed in the sediment, dorsal surface upward, somersaulted and scraped the surface with the second antennae, then burrowed again and scraped again. This species has the best-developed urosome carina of the groups, indicating that much burrowing and other movements in sand are important activities in its life.

The few ecological observations made on distribution of European species agree moderately well with the three divisions outlined here. Much more work on ampeliscid feeding methods is needed, however, before their validity

is fully established. The three groups do indicate the correlation between feeding method and morphology in ampeliscids and help to make clear that the reason for the extensive development of thickened carina or boss on the urosome of some species is the mechanical advantage it gives in coarse sediments. The difference between *A. vadorum* and *A. abdita* is particularly revealing. *Ampelisca vadorum*, which occurs on coarse sand sediments, has a well-developed boss or carina, whereas the fine sand- and mud-dwelling *A. abdita* has a slightly developed carina even in the largest adults. Mysteries which remain unsolved, however, are the adaptive reasons for the extensive variation in head shape and the form of peraeopod 5 from species to species in the genus *Ampelisca*. A closer study of functional morphology would throw light on this subject.

Very little is known of the phylogeny of amphipods, so even less is known of the phylogeny of feeding behavior. Since Stebbing (1906), many students of the Order have accepted the family Gammaridae as the basic group, and indeed the Gammaridae is the only family certainly represented in the fossil record, though only as early as the Eocene (Dr R.R. Hessler, personal communication). If the family Gammaridae is the group from which other amphipods were derived, the basic feeding method was undoubtedly browsing, using mouthparts and gnathopods to manipulate food. From this generalized feeding method a considerable adaptive radiation has occurred, and a number of groups have evolved forms of fine particle feeding independently. Most of these groups make at least some use of the pleopod current in feeding, and have set up additional currents to concentrate food in various regions. In the haustoriid *Haustorius arenarius* (Dennell, 1934) the second maxillae have a rotatory movement and act as a filter to remove particles sucked in by their own movements. Marginal setae on the inner and outer plates of both sides form the actual filter. Dennell (1934) looks on this feeding method as a modification of the "raptorial" feeding of Gammaridae.

Watkin (1939b, 1940) analyses swimming and burrowing in the haustoriids *Bathyporeia* and *Urothoe*, where food is apparently caught on a mid-ventral screen created by the setae of the second gnathopods, and the main feeding current is entirely that of the pleopods. This is also one of the feeding methods observed in the tube-dwelling corophiid *Corophium volutator* by Hart (1930).

The observations of Enequist (1950) and myself indicate that a quite different method is utilized in the ampeliscids, where the pleopod current is secondary to that created by the second antennae (in the "whirling" method) or has no food-gathering function (in the "passive-sifting" and "scraping" methods). Ampeliscid feeding methods are unique in the amphipods.

Enequist (1950) has analyzed feeding in several other families as well, illustrating the adaptive radiation within the order. When such observations are expanded into functional morphological studies and are combined with the present systematics, the ground will be prepared for the first rational phylogeny of the amphipods.

REPRODUCTIVE BEHAVIOR

A suggestion that reproduction occurs at times of full moon and spring tides in *A. abdita* was put forward previously. This idea was based on the bimodality of each generation as shown in length-frequency histograms (Fig. 2). Field evidence was obtained on July 16, 1962, when I took a number of night plankton tows in Barnstable Harbor under a full moon. Large numbers of bottom invertebrates, including trilobite larvae of *Limulus polyphemus* Linnaeus, adults of *Monoculodes edwardsi* Holmes, and many adults and juveniles of *A. abdita*, were swimming freely in the water. On the daytime high tide of the following day I could find none of these species. Russell (1925) noticed a similar phenomenon in the English channel. Night plankton samples included a number of benthic amphipod species in the genera *Monoculodes*, *Bathyporeia*, and *Ampelisca*, whereas day samples did not. Fish (1925) noted 27 species of gammaridean amphipods in the Woods Hole plankton, including *A. compressa* Holmes, *A. "macrocephala," A. "spinipes,"* and *Byblis serrata* Smith. I have examined a nighttime surface collection of *A. abdita* taken by V. N. Edwards at Woods Hole, Mass. on May 2, 1888 (now in the United States National Museum). Although both *A. vadorum* and *A. abdita* occur in the immediate area, this large collection contains only *A. abdita*, indicating that the stimulus for swimming must be different or have different timing in the two species.

Watkin (1939a) noted a lunar periodicity in the pelagic occurrence of *Bathyporeia* species near Millport, Scotland. In this genus the period was about 15 days, indicating a correlation with both new and full moon rather than just full moon. Some free swimming occurred almost every night, but the incidence increased greatly at new and full moon. Watkin (1939a) felt that the periodicity was correlated with reproduction in the adults and not with feeding, since mating and release of young occurred at these times. Young animals matured in 15 days, so free swimming of adults every 15 days ensured dispersal of the juveniles. Several species of the genus *Bathyporeia* in the area remained separated by emerging at different stages of the tide.

The phenomenon in *A. abdita* appears quite similar, although the greatest peaks of free swimming (inferred from length-frequency data) appear to be 20 to 30 days apart. This suggests that the effect of moonlight is most important, although some animals are undoubtedly found free swimming most nights. The collections were quite remarkable in containing large numbers of animals at all stages of development, from small juveniles to fully adult males and females, and they were taken when extremely dense concentrations of adults occurred in extensive patches on the low tide flats. Immediately after July 16, these dense concentrations had begun to disappear as tubes washed out. Most tubes in this area were empty. By July 21, tiny tubes of young animals became abundant everywhere, especially along the low tide channels in areas other than those previously inhabited.

These observations suggest that the free-swimming period has two important functions. First, it allows adults to meet in the water column and mate.

Second, it causes ovigerous females to be scattered into previously unoccupied areas where they will settle into the sediment and release their young. The release of young accounts for the great number of small tubes observed by July 21, and the movement of females accounts for the depopulation of old areas and the quick appearance of newly populated ones. This move is an extremely important one for the juveniles since it allows them to begin free life in an area not fully occupied by feeding adults. Competition for food and space is reduced by this method. In addition, old areas have become unstable due to accumulation of silt and fecal material, and tubes begin to wash out under heavy current conditions. Movement allows the population of juveniles to build anew on fresh unmodified sediments.

A similar shifting of centers of population was noted by Watkin (1941) in intertidal populations of *Corophium volutator*. A great depopulation of old areas occurred at breeding time. Watkin attributed the change partly to drying of the sediment, but it seems equally likely that a breeding dispersal could account for the depopulation, especially since Watkin suspected that mating took place in the water column in this species. An analogous phenomenon occurs in *Spisula* (= *Mactra*) *subtruncata* on the Dogger Bank (cited in Thorson, 1957, p. 484). Areas where adults are present become silty and unfavorable for larvae, probably because of both competition and unsuitable sediments. The larvae of each year are successful in new areas and the result is patches of the species, each containing only one year-class. This phenomenon, the shift of populations within relatively small areas, with the result that intraspecific competition is reduced and more suitable sediments are occupied, has apparently not been given much attention before.

DISTRIBUTION: TEMPERATURES, DEPTHS, AND SEDIMENTS

Historical factors governing the present distribution of *A. vadorum* and *A. abdita* have been considered in another paper (Mills, 1965), and the physical and biological determinants will be discussed here.

Broadly speaking, both species occur in the temperate waters of eastern North America and are unknown from Central America, the Caribbean region, and extreme southern Florida. Their range encompasses a number of the faunal provinces outlined by Hedgpeth (1953, 1957), the Boreal from Nova Scotia to Cape Cod, the Virginian from Cape Cod to Cape Hatteras, and the Carolinian extending from Cape Hatteras to the western Gulf Coast, with a break in southern Florida. To the north, the range of *A. vadorum* extends into the water masses referred to by Dunbar (1953, 1955) as "subarctic," that is, water of rather low temperature which extends at times as far as southwestern Nova Scotia. Whatever other utility these zoogeographical and oceanographic units may have, they do show that these species occur in fairly wide ranges of temperatures and faunal associations and must be quite eurythermal in their growth and reproductive processes.

Ampelisca vadorum has been recorded as far north as St. Simon, New Brunswick, near the south shore of the Baie de Chaleur, whereas the northernmost record of *A. abdita* is the Sheepscott River estuary, Maine. At the northern end of its range, *A. vadorum* can exist only in warmer areas, such as the shallow inner waters of St. Simon, New Brunswick, the warm bays of Prince Edward Island, the shallow lagoonal waters of the Magdalen Islands, and the shallow harbor of Cheticamp, Cape Breton Island, Nova Scotia. A number of other warm-temperate invertebrates, including the American oyster *Crassostrea virginica* (Gmelin), have similar distribution as observed by Bousfield (1958, 1962). *Ampelisca abdita* apparently does not occur to the north beyond the Sheepscott River estuary, Maine, suggesting that it has less ability to tolerate low temperatures than does *A. vadorum*.

Parr (1933) clearly demonstrated the presence of a temperature barrier at Cape Hatteras in the winter, with relatively homothermal water stretching northward from that area to the Gulf of Maine. South of Cape Hatteras in winter, shallow water temperatures will be 10 C or more, whereas to the north temperatures go much lower. Orton (1920) pointed out that the primary factor controlling breeding in many marine animals was not food but temperature, and that many species must be brought above a minimum temperature, a "physiological constant" of the species, before breeding begins. Southern races, then, will breed earlier and longer than northern ones, and the size attained by any species will depend on the relative lengths of breeding and non-breeding periods. This appears to be exactly the case in *A. vadorum* and *A. abdita*, as pointed out before. South of Cape Hatteras, breeding continues throughout the winter, whereas to the north it ceases about September and is not resumed until water temperatures go above 8 to 10 C in April. The long non-breeding period of overwintering animals north of Cape Hatteras results in a considerable size difference between the two generations in both species (Table I). However, south of Cape Hatteras, where the two generation phenomenon does not occur there is no breakdown of the size distinction between the two species even where they co-occur, clearly indicating a genetic difference in growth and development.

At the northern limit of the range of *A. vadorum* temperatures do not reach 10 C until June in most areas (Lauzier et al., 1957). This may allow time for only one generation per year, breeding in June and July, juveniles then overwintering until the following summer. The absolute northern range, the south shore of the Baie de Chaleur, and the Magdalen Islands appears to be the result not of temperatures too low for breeding, since temperatures above 10 C are found in several areas of the northern Gulf of St. Lawrence and in southern Newfoundland, but of some other temperature effect. The warmest summer temperature at the extreme northern range is apparently 16 C and this temperature is probably critical for suvival of juveniles, since Kinne (1954b, 1959) shows that juveniles of *Gammarus duebeni* are much more temperature sensitive than adults. Temperatures at the northern end of the range may become too low to allow summer repopulation. If this is the case, *A. vadorum* and probably

A. abdita, fit Hutchins' (1947) Type 2 or 3 distributions, governed in poleward range by summer temperatures high enough to allow repopulation and in equatorward range by winter temperatures low enough to allow repopulation (Type 2), or summer temperatures low enough to ensure survival (Type 3). Although a summer temperature allowing repopulation is undoubtedly the northern range determinant, the factors governing the southern range of both species (southcentral Florida and the Gulf Coast west at least to the Mississippi delta) are unknown. Orton (1920) suggested that at the extremes of ranges physiological races might evolve as adaptations to the temperature regime. This could be the case with the Gulf of Mexico form of *A. vadorum* (mentioned in Mills, 1964a, p. 569). If so, the Gulf of Mexico should be the southern limit of both species. Until more shallow water collecting is done along the Gulf Coast of Texas and Mexico and in the West Indies the exact southern limits of distribution will be uncertain.

Only three *Ampelisca* species, *A. vadorum*, *A. abdita*, and *A. "holmesi"* Pearse are endemic to temperate eastern North American waters (see Mills, 1963a). A number of northern European species are restricted to colder and deeper waters of eastern North America. Hutchins (1947) pointed out that most European invertebrates will be cold-stenothermal in their preferences, due to the uniform temperature regime from central Norway to West Africa, and thus will be restricted to cold areas and deeper waters in the western Atlantic. The most wide-ranging of these European-Northern Hemisphere species is *A. macrocephala*, known to occur as far south as Cape Cod. This species has a circumpolar distribution, and is common in the Arctic (Stephensen, 1925; Dunbar, 1954). Its occurrence on the Pacific coast of North America (Barnard, 1954, 1960) and into warmer waters of the western Atlantic suggests that a critical examination of the species should be undertaken. The morphologically similar *A. eschrichti* Kröyer occurring from 10 to 300 m in European waters is known from 60 to 256 m off eastern Canada, and occurred as far south as George's Bank in 1872 (Mills, 1964b) at a depth of 82 m. The increase of up to 5 C in New England waters over the last 30 years (Taylor et al., 1957) may have forced the species northward. Shoemaker (1931) records *A. aequicornis*, *A. amblyops*, and *A. gibba* at 212 m off eastern Canada, whereas their depth ranges in the eastern Atlantic are 10 to 100 m, 200 to 1096 m, and 60 to 3200 m respectively. Shoemaker (1931) mentions the occurrence of *A. typica*, found at 10 to 120 m in European waters, but gives no depth information. An undescribed species, erroneously referred to as *Ampelisca spinimana* by Mills (1963a) has now been recognized from the continental slope of North America. In Cabot Strait, where it has been recorded at 378 m, temperatures never go above 5 C (Lauzier and Bailey, 1957).

The records just mentioned (mostly derived from Stephensen (1925) for Europe and Shoemaker (1931) for North America) show that continuity with the European shallow- and deepwater fauna occurs around the northern North Atlantic, but, as expected, these species have not been able to invade the shallow waters of eastern North America, leaving room for North

American species which occur in shallow water. *Ampelisca vadorum* goes from the intertidal zone to 70 m (Mills, 1963a), whereas *A. abdita* is known from the intertidal zone to 27 to 29 m (Sanders, 1956).

The sediment composition for a number of localities where one or both of the sibling species occur is summarized in Table VIII. A casual inspection shows that *A. vadorum* tends to occur on coarse sand areas, often where broken shell concentrations are high (station 1, Long Island Sound) and *A. abdita* is most common on fine sand to silt without shell. The shell component cannot be essential to *A. vadorum* however, since it is very low at station 4, Long Island Sound.

Where sediments are finer and better sorted (station P, Buzzards Bay) both species occur, although in low numbers. At station P, sediments are very well sorted, with over 90% in the narrow range 0.10–0.50 mm. A noticeable shell fraction is also present. Sand grains of 0.18 mm are most easily moved by currents (Sanders, 1958), indicating that station P is a region of relatively minor currents and stable sediments. Such areas are always characterized by well-developed infaunas (Sanders, 1958). Barnstable Harbor is another area with well-sorted sediments and a well-developed infauna. Table VIII shows that the only major difference between station P and the Barnstable flat is the percentage of fine sand sediments. Apparently where a major proportion of the sediment falls between 0.05 and 0.25 mm, *A. abdita* is favored. Densities in such areas may be 4100 to 73,000/m². Where a major proportion of the sediment falls between 0.10 and 0.5 mm, both species may occur, though in relatively low densities, and other ampeliscids are far more abundant. Where a large proportion of the sediment is in the 1.0 to 2.0 mm range, only *A. vadorum* can occur.

Station 5, Long Island Sound, shows an unusual combination of sediment sizes with 18.5% of the sediments in the silt range and a large percentage as large as 1.0 mm. Only *A. abdita* was taken in this area, indicating that it can occur on relatively coarse sediments where a considerable fine fraction is also present. Where the fine fraction is absent and coarse sediments rather than fine sands are extensive, only *A. vadorum* can occur. The only species able to exist in areas of deposition where silts predominate is *A. abdita*. In such areas its relatively larger gill area must be of considerable advantage. On coarser sediments, where currents are stronger, the more robust build of *A. vadorum* allows it to burrow, build tubes, and feed more effectively than *A. abdita*.

From the data on sediment occurrences and densities of the two species it is difficult to draw any conclusions about the possible competitive interactions. Since both species are relatively scarce at station P, Buzzards Bay, where they co-occur, and since only *A. abdita* occurs in Barnstable Harbor and is extremely abundant there, some form of mutual inhibition or competition may reduce the numbers of both species where they co-occur. However, it is more likely that both species find station P physically unfavorable and both can maintain only small populations there, whereas other ampeliscids may

TABLE VIII. Sediments as percentage by weight and species present at various localities in Long Island Sound and Cape Cod regions. LIS — Long Island Sound (see Sanders, 1956, for localities); BB — Buzzards Bay (see Sanders, 1958, for localities); | — includes 90% of sediment classes.

	Grain size (*mm*)	Sta. 4 LIS	Sta. 1 LIS	Sta. P BB	Barnstable	Sta. 5 LIS	Sta. 3 LIS	Sta. R BB
Gravel	>2.0	16.80	25.71	0.06	0	2.63	0	0.20
Very coarse sand	2.0–1.0	10.62	4.86	0.04	0.94	3.42	0.35	0.12
Coarse sand	<1.0–0.5	25.52	21.45	0.32	0.53	28.64	1.36	0.28
Medium sand	<0.5–0.25	18.98	14.70	12.50	1.06	18.97	1.49	0.84
Fine sand	<0.25–0.10	22.84	25.14	78.05	32.20	27.98	10.09	1.36
Very fine sand	<0.10–0.05	0.74	0.36	4.04	58.36	0.55	12.97	5.27
Coarse silts	<50–20u	1.18	1.35	1.33	1.56	4.64	18.05	37.32
Medium silts	<20–5u	0.58	0.70	2.78	1.16	2.58	11.30	24.06
Fine silts	<5–2u	0.58	0.31	0.52	0.77	1.55	6.00	12.22
Very fine silts	<2u	3.35	2.08	0.38	3.42	9.71	34.85	18.18
Shell		0.19	10.16	Present	0	Present	0	0
Ampelisca species present		*Vadorum*	*Vadorum*	*Vadorum Abdita*	*Abdita*	*Abdita*	*Abdita*	*Abdita*
Ampelisca density /m²		1,885	1,307	low	4,100–73,000	1,360	46	Low

become abundant (e.g. *Ampelisca "holmesi"* and *Byblis serrata*). *Ampelisca abdita* is generally favored in warm shallow waters, whereas *A. vadorum* occurs deeper. Thus at Barnstable *A. abdita* is able to reach great densities, and this abundance suggests that such an environment is close to optimal for the species. Sanders' (1956) data suggest that *A. vadorum* reaches greatest densities in an environment such as that at station 4, Long Island Sound, although its densities are always lower than those attained by *A. abdita* in its suitable environments.

When both species are present, as at station P, Buzzards Bay, the taxonomic problems involved in separating the two species become important. Sanders (1958) correctly suggested that overlap of distribution occurred in Buzzards Bay, making the separation of the two groups more difficult than he found it in Long Island Sound. More widespread sampling in Long Island Sound, combined with the present knowledge of the taxonomy of the two species, would undoubtedly show that similar ecological overlaps occur on intermediate sediments in Long Island Sound and that population densities of the two species are always low in such areas.

Factors governing the local distribution of *A. vadorum* within its optimal environment are unknown. Since this species is apparently subtidal, or at best reaches only the extreme lower margin of the intertidal zone, sediment composition may be the main factor. Intertidal populations of *A. abdita* are governed in their distributon by other factors in addition to sediment size. At Barnstable Harbor this species is found along the edges of deeper channels, only extending onto the exposed flats in local depressions where the sediment remains moist or where pools of water stand at low tide. No obvious sediment changes are associated with this distribution, so it seems most likely that the species occurs only where it will not remain exposed for long, and that local distribution is a result of sensitivity to dessication or high temperatures. In deeper waters distribution at the local level is probably correlated with sediments, as discussed previously.

ASSOCIATED FAUNA

Common species associated with *A. vadorum* and *A. abdita* are listed in Tables IX–XII. In Table XI, summer 1959, data are from Sanders et al. (1962); summer 1960, data are from Sanders' unpublished work; and January 1962, data are derived from my own collections.

The main facts about the species present have been reviewed by Sanders (1958). Sand sediments (station 1 and station P) have a relatively high percentage of filter feeders and epifauna, whereas muddy sediments (station 3) have the greatest numbers of infaunal deposit feeders. Barnstable Harbor, being somewhat intermediate in sediment type, has a mixed population of infaunal deposit feeders, filter or detritus feeders, and epifaunal browsers.

A comparison of the various stations shows that stations of similar sediment type have numerous faunal elements in common. Station 1 (Long

TABLE IX. Common species and their numbers per square meter at station 1, Long Island Sound (from Sanders, 1956).

	No./m²
Coelenterata	
Hydroids	–
Polychaeta	
Lepidonotus squamatus (Linnaeus)	68
Neanthes succinea (Frey and Leuckart)	281
Nephtys incisa Malmgren	91
Nephtys caeca (Fabricius)	236
Glycera americana Leidy	30
Ampharete acutifrons (Grube)	236
Hydroides dianthus (Verrill)	61
Crustacea	
Neomysis americana (S. I. Smith)	190
Ampelisca vadorum Mills	1307
Parametopella cypris (Holmes)	699
Podoceropsis nitida (Stimpson)	46
Unciola irrorata Say	91
Erichthonius brasiliensis Dana	471
Corophium acherusicum Costa	1543
Aeginella longicornis (Kröyer)	23
Edotea montosa (Stimpson)	23
Crangon septemspinosus Say	91
Pagurus longicarpus Say	84
Pagurus pollicaris Say	53
Pelia mutica (Gibbes)	8
Neopanope texana sayi (S. I. Smith)	68
Panopeus herbsti Milne-Edwards	144
Mollusca	
Nucula proxima (Say)	8
Mulinia lateralis (Say)	471
Crepidula plana Say	334
Eupleura caudata (Say)	46
Mitrella lunata (Say)	8
Nassarius trivittatus (Say)	23
Acteon punctostriatus (C. B. Adams)	8

Island Sound), with coarse sand sediments, is quite similar to station P (Buzzards Bay), except that the slightly finer sediments at station P allow the occurrence of *A. abdita* and a better developed polychaete and mollusc infauna. Station 3 (Long Island Sound) is closely paralleled in its common species by station R (Buzzards Bay) and Sanders (1958) concluded that his *Nephthys incisa-Yoldia limatula* community occured in these two areas, although *Nucula proxima* became a more common element in Buzzards Bay. A community somewhat similar to this has been described from Greenwich Bay, Rhode Island by Stickney and Stringer (1957). A number of the common species at station R occur in muddy areas of Greenwich Bay, including "*Ampelisca spinipes*" (undoubtedly *A. abdita*). Other elements of the fauna indicate that a considerable sand or fine sand component must also be present in some areas of Greenwich Bay, and it is possible that both *A. vadorum* and *A. abdita* occur in these areas, as in some areas of Buzzards Bay.

TABLE X. Common species of the Buzzards Bay *Ampelisca* association (e.g. station P) in order of abundance within each group (from Sanders, 1958).

Polychaeta
- *Glycera americana* Leidy
- *Nephtys bucera* Ehlers
- *Ninöe nigripes* Verrill
- *Lumbrinereis tenuis* (Verrill)
- *Nephtys incisa* Malmgren
- *Sthenelais boa* (Johnston)
- *Pholoë minuta* (Fabricius)
- *Eteone lactea* (Claparède)
- *Phyllodoce arenae* Webster
- *Paraspionosyllis longicirrata* (Webster and Benedict)
- *Podarke obscura* Verrill
- *Nereis zonata* Malmgren
- *Diopatra cuprea* (Bosc)
- *Scoloplos fragilis* (Verrill)
- *Pista* sp.
- *Polycirrus eximius* (Leidy)
- *Ampharete arctica* Malmgren
- *Scalibregma inflatum* Rathke

Crustacea
- *Ampelisca vadorum* Mills
- *Ampelisca abdita* Mills
- *Ampelisca "macrocephala"* Kröyer
- *Byblis serrata* S. I. Smith
- *Unciola irrorata* Say
- *Oxyurostylis smithi* Calman
- *Paraphoxus spinosus* Holmes
- *Phoxocephalus holbölli* Kröyer
- *Leptocheirus pinguis* (Stimpson)
- *Cerapus tubularis* Say
- *Corophium* spp.
- *Ptilanthura tenuis* Harger
- *Edotea montosa* (Stimpson)
- *Stenothöe* sp.
- *Batea catharinensis* Muller
- *Erichthonius brasiliensis* Dana
- *Aeginella longicornis* (Kröyer)
- *Crangon septemspinosus* Say
- *Pagurus annulipes* Say
- *Neopanope texana sayi* (S. I. Smith)

Mollusca (Bivalvia)
- *Cerastoderma pinnatulum* (Conrad)
- *Tellina agilis* Stimpson
- *Nucula delphinodonta* Mighels and Adams
- *Pandora gouldiana* (Dall)
- *Lyonsia hyalina* (Conrad)
- *Laevocardium mortoni* (Conrad)
- *Ensis directus* (Conrad)

Mollusca (Gastropoda)
- *Natica pusilla* Say
- *Nassarius trivittatus* (Say)
- *Crepidula plana* Say
- *Mitrella lunata* (Say)
- *Anachis avara* (Say)

Tunicata
- *Molgula complanata* Alder and Hancock

TABLE XI. Barnstable Harbor *Ampelisca* association, numbers per square meter.

	Summer 1959	Summer 1960	January 1962
Platyhelminthes			
"White turbellarian"	0	500	0
Anaperus sp.	0	400	0
Rhyncocoela			
Micrura leidyi (Verrill)	2	0	0
Micrura sp.[b]	4	3	150
Cerebratulus lacteus (Leidy)	0	1	0
Amphiporus sp.	1	10	0
Polychaeta			
Eteone heteropoda Hartman[b]	200	580	550
Nereis arenaceodonta Moore[b]	23	220	1400
Glycera dibranchiata Ehlers	0	1	0
Lumbrinereis tenuis (Verrill)[b]	11	280	1800
Scolelepis squamata (O. F. Müller)[b]	80	7050	150
Polydora ligni Webster[b]	27	40	500
Prionospio heterobranchia Moore[b]	0	40	1000
Pygospio elegans Claparède[b]	100	520	700
Streblospio benedicti Webster[a]	5200	2950	2250
Tharyx sp.[b]	100	2330	300
Heteromastus filiformis (Claparède)	196	570	200
Amphitrite ornata (Leidy)	0	0	150[d]
Polynoidae	0	0	50
Diopatra cuprea (Bosc)	2	0	0
Scoloplos robustus (Verrill)[a]	11	0	0
Scolecolepides viridis (Verrill)	8	0	0
Clymenella torquata (Leidy)	1	22	[c]
Lepidametria commensalis Webster	0	5	0
Phyllodoce groenlandica Oersted	0	5	0
Aricidea sp.	0	190	0
Pectinaria gouldi (Verrill)	0	2	0
"Unknown polychaetes"	0	20	0
Ostracoda	0	100	0
Oligochaeta	[c]	1000	[c]
Crustacea			
Oxyurostylis smithi Calman	1	550	0
Leptochelia savignyi (Kröyer)[b]	0	0	150
Edotea montosa (Stimpson)[b]	30	20	150
Ptilanthura tenuis Harger	0	10	0
Ampelisca abdita Mills[b]	10	100	43,200
Gammarus sp.	19	0	50
Hyale sp.	0	0	100[d]
Carinogammarus mucronatus (Say)[a]	37	20	0
Monoculodes edwardsi Holmes	1	200	0
Ampithoe rubricata (Montagu)[a]	10	0	0
Corophium insidiosum Crawford[a]	3	0	0
Corophiidae[a]	16	0	0
Crangon septemspinosus Say	3	80 (small)	0

(*Continued*)

TABLE XI. Barnstable Harbor *Ampelisca* association, numbers per square meter.—(*Concluded*)

	Summer 1959	Summer 1960	January 1962
Mollusca			
Mytilus edulis Linnaeus	3	20	100[d]
Gemma gemma (Totten)[b]	300	300	27,400
Mya arenaria Linnaeus	0	0	50[d]
Ensis directus (Conrad)	1	5	0
Tellina agilis Stimpson[a]	16	230	0
Aligena elevata Stimpson	1	4	[c]
Mercenaria mercenaria (Linnaeus)	0	30	0
Solemya velum	0	1	0
Hydrobia minuta (Totten)[a]	75	0	50
Nassarius obsoletus (Say)[a]	209	0	0
Retusa canaliculata (Say)[a]	4	0	0
Polinices duplicatus (Say)	0	1	0
Crepidula convexa Say	0	10	0
Echinodermata			
Leptosynapta tenuis (Ayres)	0	11	0
Hemichordata			
Saccoglossus kowalevskyi (A. Agassiz)	0	380 (small)	0

[a]Species markedly less abundant in 1962 than in 1959.
[b]Species markedly more abundant in 1962 than in 1959.
[c]Numbers uncertain.
[d]Sampling error because of patchy distributions.

TABLE XII. Common species and number per square meter at station 3, Long Island Sound (from Sanders, 1956).

Coelenterata	
Cerianthus americanus Verrill	30
Polychaeta	
Nephtys incisa Malmgren	106
Lumbrinereis tenuis (Verrill)	23
Pectinaria gouldi (Verrill)	15
Unidentified polychaete	213
Crustacea	
Ampelisca abdita Mills	46
Mollusca	
Nucula proxima (Say)	1984
Yoldia limatula (Say)	61
Pandora gouldiana (Dall)	8
Pitar morrhuana (Linsley)	8
Mulinia lateralis (Say)	8
Retusa canaliculata (Say)	167

My main work on the fauna associated with *Ampelisca* concentrations has been at Barnstable Harbor, where a well-developed fauna occurs with high densities of *A. abdita* (see Table XI, January 1962, data). This area was studied extensively in 1959 by Sanders et al. (1962) before *A. abdita* was present in large numbers. Sanders re-examined the area ("station E") in the summer of 1960 and found a marked change in the fauna (Sanders et al., 1962, and unpublished data). In July 1961, *A. abdita* was first noted in this area in high densities and it persisted as a dominant organism of the area at least until midsummer 1963. The mechanism of the change is suggested by the faunal data for 1959, 1960, and 1962 and by other observations made on the flat in 1961 and 1962. A number of physical and biological features of the flat before and after colonization by *A. abdita* are compared in Table XIII.

In 1959 the Barnstable flat was characterized by a low number of individuals per square meter, a fairly high number of species, median grain size falling in the fine sand category, low silt-clay percentage and low chlorophyll concentration (see Sanders et al., 1962, Table II for comparison with other areas of Barnstable Harbor). The most noticeable feature of the flat in 1959 was the presence of large aggregations (209/m² or more) of the mud snail *Nassarius obsoletus* (Say). These snails emerge from deeper water in the spring and lay eggs on this flat in early summer. Large numbers of snails remain on the flats until late summer, moving about over considerable areas. A study completed in another area of Barnstable Harbor (Grant, 1965) reveals that the arrival of *Nassarius* in great densities on a flat results in a sudden reduction in the numbers of other species. This reduction is sharp enough to be attributed to an antibiotic effect, but could equally be due to reduction of food supplies by *Nassarius* grazing or to simple mechanical disturbance of the substratum, since the snails plow along just below the sediment surface, leaving noticeable tracks and much upturned sediment.

The sudden success of *A. abdita* and the marked faunal changes shown in Table XI are probably due primarily to the activities of *Nassarius*. The mechanism of the change could be as follows.

Nassarius fed on this part of the Barnstable flats regularly in summer, grazing benthic diatoms and flagellates and disturbing the substratum usually inhabited by a well-developed mollusc and polychaete fauna (mostly the lamellibranch *Gemma gemma* and spionid polychaetes). The remarkable change in the fauna of this area from 1959 to 1960 (noted in Sanders et al., 1962) was due to disturbance by *Nassarius*, followed by colonization by a number of species in the unstable area. Low chlorophyll values (217 mg/m²) suggest that *Nassarius* was grazing the area heavily. *Nassarius* left the flat in winter, allowing recolonization only by species which could breed in late summer, fall, and winter. In the following spring, breeding individuals of *A. abdita* from other areas were carried onto the flat. Since the density of other species was low the area was suitable for colonization, and with the beginning of summer breeding in June and July a spectacular increase in

TABLE XIII. Measurements on the Barnstable, Massachusetts *Ampelisca* flat.

Physical and biological factors of the Barnstable *Ampelisca* flat (1959 data from Sanders et al., 1962).

	No./m²	Min. biomass	Species no.	Wt suspension feeders (g)	Wt deposit feeders (g)	Wt carnivores (g)	Median grain size (*mm*)	Silt-clay (%)	Organic (%)	Chlorophyll (*mg/m²*)
1959	7,000	25.8	37	0.6	24.5	0.7	0.14	5.54	0.87	217
1962	80,450	–	22	–	–	–	0.074	6.91	–	355–799

Chlorophyll a values on the Barnstable *Ampelisca* flat, mg/m²

1959	217 mg/m²	
1962	1. Tubeless sandy depressions:	354.7±97.5 (standard deviation)
	2. Tube areas, including tubes:	798.5±155.1

numbers occurred. This increase was made possible by the absence of heavy competition from filter feeders such as *Gemma gemma* and the selective deposit feeders *Streblospio benedicti*, *Pygospio elegans*, and *Prionospio heterobranchia*. Competition for space, if not for food, must occur in associations of these species with *A. abdita* since every conceivable area is occupied by tubes. When the animals are feeding the whole surface of the sediment is covered by the moving tentacles of spionids, beating second antennae of *Ampelisca*, or by the intake of water to *Gemma* siphons. The whole biology of *A. abdita*, including the steep, linear growth curve, short incubation period of the eggs, and movement of centers of density suggests intense competition for space.

With the establishment of densities of *Ampelisca* tubes, *Nassarius* found the sediment in these areas unsuitable and then aggregated only where tubes were absent. When *Ampelisca* became well established, a number of other species (marked as footnote b in Table XI), undoubtedly including some partial competitors, were able to increase in numbers since a stable substratum for tube-building and feeding had been formed by *Ampelisca* tube-building. Selective deposit-feeding polychaetes were the main species favored, including *Eteone heteropoda*, *Nereis caudata*, *Lumbrinereis tenuis*, *Nerinides agilis*, *Polydora ligni*, *Prionospio heteorbranchia*, *Pygospio elegans*. and *Tharyx* sp. as well as the filter-feeding lamellibranch *Gemma gemma*, which became extremely abundant. The isopod *Edotea montosa* found the complex surfaces of tubes and sediment very favorable for browsing on detritus and dead animal material. The only small polychaete which did not increase in number with the establishment of *A. abdita* was *Streblospio benedicti*. This species is probably a direct competitor with *A. abdita*. Its mode of feeding may be disturbed by the presence of *Ampelisca* tubes since *S. benedicti* feeds by moving its palps over the surface of a relatively level substratum. Where conditions are favorable it may reach densities of over 41,000/m² but even under less favorable conditions of the *Ampelisca* flat the species can still maintain over 2000 animals/m².

When *A. abdita* became well established on the flat, favoring the increase of associated species, it may have improved the conditions for its own growth. This may account for the larger size of summer animals in 1962 compared with 1961 (Table I and mentioned previously). The difference could also be due to other factors such as food supply or temperature.

After its arrival on the flat, *A. abdita* brought about a considerable change in the nature of the environment. The main food material is detritus and associated fine sand grains which are consolidated in the fecal material produced and deposited around the tubes. The construction of tubes binds up fine particles and the completed tubes act as sediment catchers. As a new area of tubes develops, the area of colonization becomes noticeably softer as fine sediments accumulate (note change in median grain size, Table XIII). Activity within the tubes is sufficient to carry some oxygen well into the sediment, keeping it oxidized to the bases of the tubes. When tubes are vacated, a black reduced zone forms rapidly just below the sediment surface and the tubes

disintegrate at the base and then wash out easily. Even when animals remain in their tubes their sediment-catching activities may make the sediment unsuitable at times of high winds. Large areas are sometimes damaged by waves and wind in summer. The *Ampelisca* flat was damaged noticeably by a heavy wind storm in early March 1962, at a time when the population was very low in numbers. Even footprints across a heavily inhabited area allow currents to wash out large patches of tubes. Enough natural accidents cause breaks in a thick tube cover to allow washing out, and extensive tube areas soon come to have a hummocky appearance, thick with tubes on low elevations, with fine sand and water in depressions between the elevated areas.

Besides acting as a topographically complex habitat for other invertebrates, large patches of *Ampelisca* tubes increase the surface area available for diatom and flagellate growth. The magnitude of the increase is shown in Table XIII. Relatively low chlorophyll values still occur in tubeless depressions. The increase in chlorophyll values in areas with tubes is accounted for by the vertical surface area of tubes present, taking into account such factors as shading of one tube by another.

The areas covered by *A. abdita* at Barnstable vary considerably over a period of time. The major factor involved is the swimming phase at times of breeding combined with full moon and spring tides (see previous discussion). This movement occurs just when densities are very high. On July 7, 1962, just before the summer breeding began, I estimated that several large *Ampelisca* patches covered 3517 m^2 and that over 199 million animals were present in this relatively restricted area. A week later this heavily occupied area was beginning to wash out. Even subtidally a similar phenomenon is likely to occur if mating takes place in the water column, since females are not likely to find their way back to their original areas. Stickney and Stringer (1957) felt that the subtidal population in Greenwich Bay, Rhode Island, was stable in extent from year to year, but it is more likely that densities vary considerably there, if only on a relatively local scale, with each breeding period.

Storms undoubtedly affect populations considerably. The effect of a wind storm in March 1962 was mentioned previously. In Barnstable, greatest damage would occur at mid tide when waves could break directly over the inhabited areas. Ice pans occasionally fill Barnstable Harbor, but their effect on the fauna of the flats may not be great. On February 16, 1962, the harbor was full of ice, most of it pans 3 ft thick. The only signs of damage were grooves across the flats, some about a quarter of an inch deep. There was no evidence of great disturbance of the sediment. Population densities of *A. abdita* were low at this time and tubes were widely spaced, so ice would have a minimal effect.

Effects of predation are difficult to observe, but mortality due to bird feeding must be heavy at times. In winter, large flocks of ducks (mostly Black Ducks, but with numbers of Common Eiders and Pintails), Canada Geese, and Herring and Ring-billed Gulls, feed on the *Ampelisca* flat. The duck and goose droppings contained mostly ground *Gemma gemma* shells

but *A. abdita* could form a less easily detected food source. In spring and early summer, shorebird predation is likely to be heavy. The peak of the shorebird migration occurs between May 15 and June 7, just at the end of the first breeding period of *A. abdita*. Least Sandpipers feed regularly in the *Ampelisca* areas, apparently picking the animals from tubes and possibly also eating the spionid polychaete *Streblospio benedicti*. A little later in the migration the Least Sandpipers are joined by Semipalmated Sandpipers. A number of other species such as Greater Yellow-legs and Black-bellied Plover feed near patches of *Ampelisca* tubes, usually in flooded depressions filled with smooth sand. Apparently only the small sandpipers (Least and Semipalmated) feed regularly in the *Ampelisca* areas, whereas other species occur in areas of different sediments. A closer study of the ecological partition in shorebird flocks on such a flat could be very rewarding in judging the intensity of predation on the infauna.

The spring shorebird migration occurs just at breeding time in *A. abdita* when females are still carrying eggs, so shorebird feeding must act as a major population limiting factor. The fall shorebird migration, beginning slowly in mid-July and extending to early December (a few Red-backed Sandpipers and Sanderlings feed near *Ampelisca* areas into December) must act to limit the numbers of overwintering animals after the July to September summer breeding period.

The *Ampelisca* area at Barnstable Harbor is very new. The nature of this animal association, characterized by fluctuating numbers and radical changes of fauna, makes terminology a difficult matter. Implicit in any definition of a "community" is the idea that communities are relatively stable on a long term basis. The physical and biological factors operating at Barnstable seem likely to result in the disappearance of *A. abdita* just as suddenly as it appeared unless the species can stabilize its own environment. Two opposing factors appear to be at work. Stabilization and increased complexity of both environment and fauna is caused by the presence of *Ampelisca* tubes and modification of the sediment by feeding. This is offset by an instability of substratum caused by breeding movements and by the accumulation of fine material in the feeding and tube-building processes.

Macfayden (1960, p. 179–180) outlines the main conceptions behind the term "community." Various definitions include the ideas of "species constancy," "dynamic stability," and "organismic unity." It is too soon to say if associations such as that at Barnstable show species constancy. Their intrinsic dynamic instability has been explored in detail. However, there is in a sense a form of "organismic unity" devoid of any metaphysical overtones. The *Ampelisca* association is marked by an increasing complexity of habitat as it becomes established. Tubes provide surfaces for algal growth. Increased food supply allows an increased density of consuming organisms. Feeding activities cause the accumulation of detritus, further increasing food supplies for some organisms. Topographical complexity allows a number of epifaunal organisms to browse and hunt in a way not possible on flat sand sediments.

This association is governed not just by physical factors (primarily sediment), as Jones (1950) suggests for benthic communities, but by biotic interactions which modify the physical environment.

Associations such as that including *A. abdita* at Barnstable Harbor and *A. vadorum* in various areas are common in several areas of the world. Thorson (1957) outlines the characteristics of a "Boreo-arctic Ampelisca community" which occurs in patches throughout other communities. In European waters Thorson states that many species of *Ampelisca* are scattered throughout the "*Syndosmya alba* community," which is characterized as follows (Thorson, 1957, p. 510): "Communities living in sheltered or estuarine areas, often with a somewhat reduced salinity and on a mixed to muddy bottom rich in organic material . . . The characterizing species have a rapid growth, attain maturity at an early age, and have a short life (either "naturally" or because eaten by predators). The standing crop will comprise the production of little more than a year or, perhaps, only a fraction of it, and the total productivity is very large. Fluctuations of the quantitative composition of the community from year to year are enormous. A species dominating one year may be absent or nearly absent and replaced by another species the next year."

Obviously associations like those including *A. vadorum* and *A. abdita* are common on a worldwide scale, and the reasons for their fluctuations and peculiar characteristics are now more clear from observations at Barnstable Harbor.

ECOLOGICAL DIFFERENCES AND THE SPECIES PROBLEM

The ecological information presented in this paper, including the information on life history and general biology of *A. vadorum* and *A. abdita*, supports the morphological evidence I have marshalled (Mills, 1963a; 1964a) that these amphipods form a sibling species pair, conforming to the definitions given by Mayr (1949, p. 151; 1963, p. 34). Mayr (1963) reviews the nature of such species and points out that the main features used to distinguish sibling species in all groups of animals may include biometry, breeding tests, habits, vocalizations, host preferences, pathogenicity, parasites, commensals, symbionts, cytology, and biochemical specificity.

In general, ". . . sibling species are a problem of developmental genetics" (Mayr, 1963, p. 57). This is the idea I have developed previously (Mills, 1964a and earlier in this paper) where I showed that the two species differ in having modified the developmental rates of various parts and by stopping growth at different points along curves of allometric growth. The strict morphological differences between the two species are very similar in magnitude to those observed in several closely related species of *Gammarus* by Kinne (1954a). This study and the study by Price (1958) on cyclopoid copepods show that many more such problems of sibling species grouping will arise in invertebrate groups when the taxonomy and ecology of widely distributed and polymorphic species become better known.

This discussion also demonstrates that *A. vadorum* and *A. abdita* differ slightly in their distribution and depth preferences (and thus probably their temperature preferences). They differ most notably in sediment preferences, with this ecological factor the best initial indicator of the other biological differences between the species.

The biological characteristics of the species are a reflection of adaptation to environment as I have stressed in discussing the adaptation of *A. abdita* to a crowded environment. Rapid development is necessary to fill the environment rapidly, but aspects of development such as egg size (that is, food available to the embryo) and the size at which juveniles become free living cannot be changed much without adversely affecting survival. Gill area, relatively larger in the small species, *A. abdita*, is correlated with the ability of the adults of this species to live on relatively anoxic fine sand and mud sediments, whereas the gill area of adults of *A. vadorum* is associated with coarser sediments and stronger currents.

Although the species differ in the biological features just listed, the ecological relationship between the species where they co-occur is still uncertain. Their mode of feeding, which usually involves gathering masses of detritus, may not allow food differentiation. It is still possible that the manipulation of food particles by the mouthparts remains a means of food differentiation. However, it is most likely, judging by their environments, that the species are seldom food-limited, that they can coexist without intense competition on some sediments, but that their individual ecological preferences usually keep the species separated.

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α-Tocopherol in Some Atlantic Fish and Shellfish with Particular Reference to Live-Holding Without Food[1]

By R. G. Ackman and M. G. Cormier[2]

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

ABSTRACT

Muscle tissue from four species of Atlantic fish (cod, dogfish, mackerel, and flounder) and one species of Pacific fish (sablefish) was examined for total lipid and α-tocopherol content. The values for α-tocopherol in both lean and fat major muscle tissues fell in the range 210–330 μg/g lipid. Lipids from cod and mackerel livers showed slightly higher values (430 μg/g lipid). These results are interpreted as the result of a lipid integration process in which the tocopherols produced by phytoplankton are redistributed with lipid through various lower life forms and small fish until similar levels are reached in the principal fats of larger fish. The metabolically active dark (lateral line) muscle of cod was twice as rich (630 μg/g) in α-tocopherol in proportion to lipid as white muscle tissue from cod. Lobster muscle was exceptionally rich (>1200 μg/g) in α-tocopherol. Examination of starved cod indicated that large amounts of α-tocopherol remained after lipid depletion. Frozen flounder fillets showed no significant amount of α-tocopherol after 4 to 8 months frozen storage.

Eight species of Atlantic shellfish were examined *in toto*. Oysters and quahogs lost their α-tocopherol after several weeks of live-holding without food. In the oysters this was accompanied by muscle weakening and an objectionable odour. Most filter feeding species in the normal environment had 1–2.5% lipid and α-tocopherol values of 300–600 μg/g lipid, but a high proportion of organic detritus in the diet of mussels from one area reduced this level. The quahogs examined were extremely low (~ 0.60%) in lipid and therefore relatively high in α-tocopherol in proportion to lipid (> 1000 μg/g). Herbivorous periwinkles and accompanying predatory dogwinkles had the highest α-tocopherol contents on a fresh weight basis (35 and 21 μg/g tissue) and high values in proportion to lipid (~ 850 μg/g).

INTRODUCTION

The tocopherols present in fish and shellfish originate in phytoplankton (unicellular algae) and in multicellular algae. They are then passed through the aquatic animal food chain by plankton eating or herbivorous species. Several tocopherols exist in terrestial plants, but marine photosynthetic organisms were considered to have only α-tocopherol until Brown (1953a) and Jensen (1966) showed that in some multicellular algae various tocopherols were present, in a few cases with α-tocopherol as a minor component.

In marine animals there is little doubt that α-tocopherol is the principal (>90%) type of tocopherol (Harris et al., 1950; Brown, 1953b; Nazir and Magar, 1964). In the present study particular attention was paid to the analyses of plankton- and algae-feeding shellfish but no significant amount of tocopherol other than α-tocopherol was detected.

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[2]Present address (M.G.C.): Department of Fisheries, Halifax, Nova Scotia.

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With the commercial application of procedures that retard bacterial activity (freezing, irradiation, antibiotics, etc.) deterioration of fishery products caused by non-bacterial factors has become increasingly important. Chief among these is rancidity, where tocopherols may play a role as antioxidants. The reports of Lambertson and Braekkan (1959) and Braekkan et al. (1963) contain most of the data available in the literature on α-tocopherol in marine animals. Species varied rather widely in α-tocopherol content of comparable tissue such as muscle, or of organs such as the liver, although some of these differences were reduced in significance if the α-tocopherol was considered in relation to fat content. In sexually mature herring the muscle of males and females did not differ significantly in fat or α-tocopherol content, but the difference in the liver values was 10-fold.

The present study reports the details of the analytical technique employed in this laboratory for study of marine animal tocopherols and its application to local fish (with one Pacific species) and shellfish. In addition to the factors such as place of origin, season, and state of sexual maturity which are obviously important, two additional factors were noted in these preliminary studies: the postmortem history of fish flesh and the effect of starvation in live-holding cod and shellfish.

EXPERIMENTAL

There are numerous variations of several basic analytical techniques for tocopherol determinations. In part these have originated in the difficulty of separating and quantitating the various tocopherols and related materials. The tendency of tocopherols to oxidize (Loncin and Jacobsberg, 1964; Plack and Bieri, 1964), particularly in alkaline media (Diplock et al., 1966), has further complicated analyses, and there is no method which does not give some small loss during analysis. The method employed in the present study for α-tocopherol is suitable for work on phytoplankton and plankton feeders, where other tocopherols might occur, and is not necessarily as rapid or as convenient as some others but has been found satisfactory after careful checking of all sources of error. Ubiquinones possibly occur in the same fractions as α-tocopherol but do not interfere with the Emmerie–Engel determination (Lambertson and Braekkan, 1959). In some fish lipids (Pennock et al., 1959; Nazir and Magar, 1964; Ikeda and Taguchi, 1966) and some shellfish (Caserta and Ghiretti, 1963; compare with present α-tocopherol results for different species) the levels for ubiquinones in micrograms per gram tissue are similar to those of α-tocopherol.

Materials and Sample Treatment

All chemicals were reagent grade or equivalent. Ethanol (96%) was refluxed for several hours over zinc and sodium hydroxide, then distilled under nitrogen. Spectro grade *n*-hexane (Fisher Scientific Co.) was used without further purification but all other solvents were distilled in glass under nitrogen before use. All steps were carried out in a nitrogen (<10 ppm O_2) atmosphere.

Sample Saponification and Extraction

Large shellfish were drained, the meat weighed, and the total lipids extracted by the method of Bligh and Dyer (1959a). A portion of the chloroform layer (5%) was used for tocopherol determination, the balance for total lipid determination. The aliquot for tocopherol determination was evaporated to dryness in a 100-ml two-necked flash (24 mm and 14 mm). After the addition of ethanol (15 ml), pyrogallol (100 mg), water (1 ml), 50% aqueous potassium hydroxide solution (1 ml), and one or two fused silica anti-bumping granules (British Drug Houses Ltd.), the mixture was refluxed for a minimum of 30 min with a gentle current of nitrogen admitted through the condenser. After the mixture cooled, the 14-mm stopper was replaced by a pouring spout to allow convenient transfer of material under nitrogen.

The cooled saponification mixture was transferred to a separatory funnel with water rinses (3 × 15 ml) and a rinse of *n*-hexane (15 ml). Extraction of non-saponifiable materials into this *n*-hexane was followed by two further extractions with *n*-hexane (10 and 5 ml respectively). The combined *n*-hexane extracts were washed several times with water (5-ml portions), dried over anhydrous sodium sulphate, and evaporated to dryness. Normally the entire sample was used in the subsequent tocopherol determination.

In most fish-flesh determinations one or more samples (total ca. 2 g) were carefully cut by scalpel from representative areas (black, belly flap, tail) of fillets (ca. 100 g). The small sample was then added to the mixture described above for direct saponification and the larger used for total lipid determination. In two cases where sample homogeniety was in doubt (lobster flesh, mackerel) total lipid extracts were prepared from large samples and aliquots taken for tocopherol determination. The meat from very small shellfish (e.g. periwinkles) was saponified directly.

Column Chrmoatography and Tocopherol Determination

The procedure followed that of Bieri et al. (1961). Activated alumina[3] (grade F-20, Aluminum Co. of America) was moistened with 10%(w/w) water[3] and stirred until homogeneous and free of particle agglomerations. To this wetted alumina (0.3 g) was added zinc carbonate (J. T. Baker Chem. Co.) (0.3 g) and Celite 545 (Johns-Manville Co.) (0.15 g). The mixture was well stirred, poured dry[3] into the column, and compacted with tapping and the application of a slight vacuum. The column was than wetted with *n*-hexane (spectroscopic grade, Fisher Scientific Co.), 1 or 2 ml being passed through as a rinse.

The column was of borosilicate glass, approximately 15 cm overall in length and 5 mm I.D. The upper end was flared to a funnel shape and the lower ended in a 7-mm inner joint. A constriction above the joint was conveniently plugged with glass wool to support the column packing. The column was mounted on a special fraction collector shown schematically in cross section in

[3]See Bieri et al. (1961) for detailed specifications.

Fig. 1. Basically this consisted of a glass cylinder with rubber stoppers at each end bored to admit suitable connections. The purpose of this arrangement was to maintain a nitrogen atmosphere, permit application of a partial vacuum to speed up column flow, and facilitate removal of the solvent from the collected fractions. The upper rubber stopper had a delivery tube through the center, with an external outer 7-mm joint, and was bent on the inside to carry the column eluant to the collection tubes. This stopper carried two tubes to admit nitrogen from a restricted supply and permit application of an aspirator vacuum. Eight additional holes were provided above the collection tubes, of a size to permit glass nitrogen blowdown tubes (disposable pipettes, Fischer Scientific Co.) to slide up out of the way during fraction collection. These were greased with silicone grease (Dow Corning) and connected to a common nitrogen manifold. The fraction collection tubes were eight 10 mm × 70-mm test tubes (capacity 3.8 ml), supported by their lips in an aluminum disc mounted on a rotating shaft passing through the center of the lower rubber stopper (silicone grease seal). This stopper also admitted two pieces of glass tubing to permit warm water to run through the lower part of the device during the blowing down of the fractions.

The sample in *n*-hexane (100 μl) was placed on the column (without vacuum) and a further 100 μl *n*-hexane was used to rinse out the delivery pipette and the walls of the column. The vacuum was then applied and when the *n*-hexane was nearly absorbed a small amount of the eluant (benzene in *n*-hexane, 1:4 by volume) was added, with further amounts as necessary. The vacuum of 50–80 mm Hg did not cause significant loss of solvent and approximately 1.5 ml of eluant was collected in each tube. After the chromatography was completed, the vacuum was broken with nitrogen, the nitrogen blow tubes lowered into the collection tubes, warm water admitted to the bottom of the apparatus, and the fractions blown dry.

The fractions were then dissolved in ethanol (3 ml) in the same test tubes and tocopherols determined by the Emmerie–Engel procedure, with the addition of 0.5% bipyridyl in ethanol (0.2 ml) and 0.2% ferric chloride in ethanol (0.2 ml), measurement of the absorption at 520 mμ (Beckman DK-2) after 3 min.

Control Experiments

Authentic α-, β- and δ-tocopherols (Distillation Products Ind.) were used to test this analysis procedure. Amounts of β- and δ-tocopherol of less than 50 μg did not pass through columns of suitable activity, and larger amounts appeared first in fractions 5 and 6. Authentic α-tocopherol appeared in fractions 2 and 3, with occasional small amounts in fraction 4.

Complete analyses, including saponification, with known amounts of α-tocopherol gave about 95% recovery. The addition of small amounts of α-tocopherol to cod flesh extracts which had been analysed the previous day also gave approximately 95% recovery of the added α-tocopherol.

The pigments (Bligh et al., 1957) in the non-saponifiable material from lobster lipid extract did not move on the column. In the case of the shellfish,

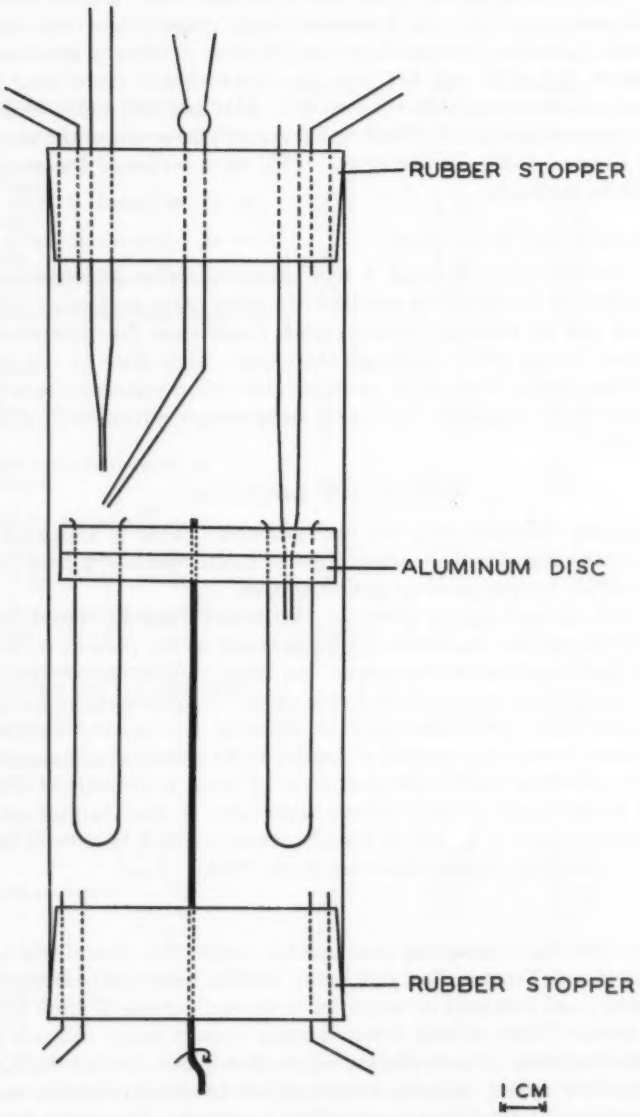

FIG. 1. Schematic view of system for collecting chromatographic eluent with vacuum-speeded flow and for rapid removal of solvent in inert atmosphere.

particularly the common mussels and periwinkles, a small amount of yellow material sometimes appeared in the first fractions. The material from mussels was found not to reduce the Emmerie–Engel reagent and was not further investigated. A similar fraction from periwinkles, in ethanol solution, showed absorption at 418, 450, and 478 mμ, the curve closely resembling that of a preparation of β-carotene (maxima at 422, 445, and 472 mμ). This material may have been zeaxanthin (cf. Bligh and Dyer, 1959b) or one of the xanthophylls (Fox and Crozier, 1965). Fisher et al. (1956) have reviewed the occurrence of carotenoids in molluscs.

Shellfish-Holding Facilities

The shellfish were received 1 day after collection and analysed either then or within 24 hr except as specified. In some cases prolonged live-holding was carried out in suitable facilities with continuous flow filtered sea water (Stewart and Power, 1963) Although there was a little slime on the inside wall of the holding tanks, there were no significant microorganisms or organic detritus in the water supplied. The water temperature varied with season from +6 C to +2 C.

RESULTS AND DISCUSSION

The results obtained with fish flesh samples (Table I) and with shellfish (Table III) are generally of the same order of magnitude as reported by Braekkan et al. (1963) for the same or similar species.

The α-tocopherol figures given by Ikeda and Taguchi (1966) for muscle in some Pacific species are similar to those found in the present study for fish with lower lipid contents in the muscle, but these authors do not give the lipid content of the tissue investigated, hence direct comparisons are not possible.

Braekkan et al. (1963) discussed the differing α-tocopherol values in some detail, but since there are a number of species in the present study not previously analysed, or analysed from a different point of view, the results of the present study will be discussed in detail where applicable. In the shellfish no account has been taken of sex or degree of sexual maturity which may be of particular importance to shellfish lipids (Bourcart et al., 1964).

Cod

All of these fish (excepting commercial sample D) were from a general purpose supply of Terence Bay cod taken in late November, December, and early January, and live-held in tanks for unknown periods of time from a few days to 3 weeks. When offered frozen herring chunks many fish will not feed under these conditions. The values for α-tocopherol may, for fish A, B, C, E, F, and G, therefore reflect to some extent unknown dietary factors as well as differing metabolic rates under non-feeding conditions. The range for α-tocopherol in white tissue (1.7–2.4 μg/g) compares very favourably with that (1.5–2.1) of Braekkan et al. (1963), but Pennock et al. (1959) report 3.3 μg/g in a cod flesh sample.

TABLE I. Lipid and α-tocopherol contents of some fish tissues and of lobster tissues.

Species and sample information	Specific fish	% lipid	α-tocopherol content: Sample (μg/g)	α-tocopherol content: Lipid (μg/g)
Cod (*Gadus morhua*)				
White tissue				
(fish taken in December, 38 cm, 0.75 kg)	(A)	0.70	1.7[a]	240[b]
	(A)	0.77	2.2[a]	290[b]
(fish taken in December, 44 cm, 0.91 kg)	(B)	0.77	2.3[a]	310[b]
(fish taken in December, 53 cm, 1.4 kg)	(C)	0.78	2.4[a]	310[b]
	(C)	0.71	2.4[a]	330[b]
(commercial frozen cod taken in February)	(D)	0.76	2.4[a]	320[b]
Dark tissue				
(fish taken in January, no other data)	(E)	1.85	11.5[a]	630[b]
	(E)	1.87	11.7[a]	630[b]
	(F)	1.83	11.6[a]	630[b]
Liver (fish taken in October, no other data)	(G)	51.2	220[b]	430[a]
Dogfish (*Squalus acanthias*)				
Dorsal muscle				
(fish 75 cm, 1.6 kg; local)	(A)	13.8	29.2[a]	210[b]
(fish 81 cm, 1.9 kg; banks)	(B)	14.5	9.2[a]	60[b]
Flounder (*Pseudopleuronectes americanus*)				
Commercial frozen fillets	(Average of 20 samples)	1.4	3.6[a]	250[b]
Mackerel (*Scomber scombrus*)				
Muscle section near tail, dark tissue (fish 27 cm, 0.48 kg)		4.9	15.2[a]	310[b]
Liver (same fish)		7.2	31.0[b]	430[a]
Sablefish (*Anaplopoma fimbria*)				
Dorsal muscle (no data)		15.9	43.5[b]	270[a]
Lobster (*Homarus americanus*)				
Claw and tail meat (110 g)	(A)	1.10	13.8[b]	1260[a]
(72 g)	(B)	0.95	11.7[b]	1330[a]
(160 g)	(C)	0.97	18.7[b]	1930[a]
Liver (same animals) (14.3 g)	(A)	21.3	15.7[b]	74[a]
(14.2 g)	(B)	21.3	12.8[b]	61[a]
(35.0 g)	(C)	–	–	–

[a]By direct analysis of tissue or lipid.
[b]Calculated value.

The dark muscle tissue was very carefully cut or scraped off fillets for examination independently of the predominant white tissue. The α-tocopherol contents are higher by a factor of 5, but the overall lipid content is higher by only a factor of 2.5. This dark tissue (lateral line muscle) is of particular interest since it is generally regarded as metabolically more active than white tissue (Braekkan, 1956; Zipser and Watts, 1961; Jonas and Bilinski, 1964; Castell

TABLE II. Data and α-tocopherol results for three cod starved 3 months.

Fish	Sex	Gonad condition	Length (*cm*)	Weights (g) Total	Liver	Other viscera
Sample data:						
H	Female	Some development	57	1400	16	72
I	Male	Little development	61	1380	29	64
J	Male	Some development	63.5	1730	35	–[a]

	α-tocopherol data			Distribution of α-tocopherol (estimated) in fish	
	% lipid	Sample (μg/g)	Lipid (μg/g)	(μg)	% of total
Flesh					
H	0.54	4.2[b]	780[c]	4100[d]	79
I	0.76	1.0[b]	137[c]	1000[d]	30
J	0.76	1.3[b]	176[c]	1640[d]	18
Liver					
H	5.2	68.5[c]	1300[b]	1100	21
I	37.8	79.5[c]	210[b]	2300	70
J	51.5	208[c]	410[b]	7260	82

[a]Not recorded.
[b]By direct analysis of tissue or lipid.
[c]Calculated value.
[d]Bone allowed at 25% of whole weight.

and MacLean, 1964). In cod it forms a significant proportion of the whole fillet only at the tail end (Fraser et al., 1961). The extractable lipid is reported as 1.88–2.06% by Castell and MacLean (1964) and 2.2% by Bligh and Scott (1966). Little variation is to be expected in the amount of lipid since it is known that the gross compositions of the light and dark tissues of cod do not differ in type of lipid, both containing essentially phospholipids with small amounts of various neutral lipids (Bligh and Scott, 1966). The higher level of α-tocopherol in the dark tissue than in the white tissue is more likely to be associated with metabolic activity than with the minor differences in types of lipid.

The liver content of α-tocopherol in one cod is also comparable with data reported by Braekkan et al. (1963) on a fresh weight basis. The triglyceride fat content of cod livers varies widely on a seasonal basis, and the values of α-tocopherol reported in cod liver oils (90–340 μg/g oil, Braekkan et al., 1963; 256–322 μg/g oil, Brown, 1953b) probably vary for this reason. In view of the wide range of values reported in the literature the present value for α-tocopherol in relation to lipid content, although high, is not excessively so.

TABLE III. Lipid and α-tocopherol contents of some shellfish when collected and after live-holding for various periods. Analyses were carried out on extracted lipid and results for samples are calculated except in periwinkles and dogwinkles.

Species, sample designation,[a] and holding period		% lipid	α-tocopherol content: Sample (μg/g)	α-tocopherol content: Lipid (μg/g)
Oyster (*Crassostrea virginica*)				
Paugh's Creek, P.E.I., July	Fresh (S)	1.27	6.0	470
	Fresh (M)	2.36	8.4	360
	Fresh (L)	2.56	11.2	450
	4 weeks (S)	1.0	2.1	200
	4 weeks (L)	0.86	4.2	480
	9 weeks (L)	0.85	Nil	Nil
	34 weeks	0.36	Nil	Nil
Paugh's Creek, P.E.I., Sept.	28 weeks	0.66	Nil	Nil
Paugh's Creek, P.E.I., Dec.	17 weeks	0.98	Nil	Nil
Bay quahog (*Venus mercenaria*)				
Paugh's Creek, P.E.I., July	2 weeks (S)	0.65	9.2	1430
	1 week (M)	0.68	11.6	1700
	1 week (L)	0.50	4.9	1000
	4 weeks (L)	0.65	Nil	Nil
Ocean quahog (*Artica islandica*)				
Pictou Island, N.S., Sept.	Fresh (P-2)	0.78	1.9	230
Horse mussel (*Valsella modiolus*)				
Smelt Creek, P.E.I., Sept.	Fresh (P-2)	1.12	5.8	530
Ribbed mussel (*Volsella demissa*)				
Smelt Creek, P.E.I., August	Fresh (P-3)	1.05	5.0	480
Blue (common) mussel (*Mytilus edulis*)				
Smelt Creek, P.E.I., August	Fresh (P-3)	1.34	5.8	430
Halifax Hbr. bottom, August	Fresh (P-6)	1.36	9.1	580
Halifax Hbr. shoreline, August	Fresh (P-6)	1.20	1.7	140
Halifax Hbr. shoreline, April	Fresh (P-6)	1.26	1.1	90
Common (edible) periwinkle (*Littorina littorea*)				
Terence Bay, N.S., Sept.	Fresh (P-10)	4.27	35.2	820
Black Rock, Halifax Hbr., April	Fresh (P-6)	4.80	35.8	730
Black Rock, Halifax Hbr., April	2 weeks (P-15)	1.21	10.6	380
Atlantic dogwinkle (*Thais lapillus*)				
Terence Bay, N.S., Sept.	Fresh (P-10)	2.45	21.2	860

[a]S,M,L = Small, medium, large; P-x = x individuals pooled.

Three cod (Table II; fish H, I, and J) were available from the December stock which were to have been starved for 3 months. When the fish were killed, two partly digested herring chunks were inexplicably found in the stomach of fish J. This was possibly an isolated case of casual tampering as all of these fish were well below minimum weights of fish of corresponding length from the same original area in a study of Terence Bay cod carried out over 1 year (Jangaard et al., 1967):

Number of fish	Sex	Length range (*cm*)	Weight of fish (*g*)		Average weight of liver (*g*)
			Minimum	Average	
7	Female	56–58	1590	1770	40
5	Male	60–62	2100	2220	57
11	Male	63–65	2160	2480	65

The percentage of fat in the livers of fish I and J did, however, fall within the normal range for the Terence Bay cod.

The fillets from fish I and J were tested for resistance to the development of rancidity (Castell and MacLean, 1964). The 24-hr TBA values were respectively 0.220 and 0.015, whereas the 24-hr TBA values with 20 ppm added copper were respectively 0.555 and 0.530. These figures indicate that the flesh of fish I was very susceptible to the development of rancidity, whereas the flesh of fish J was as resistant as some normal good quality fish despite the low level of α-tocopherol in relation to that of normal fish (Table I) taken at approximately the same time of year. The possible role of α-tocopherol in relation to susceptibility to oxidation of cod flesh at various times of the year has been discussed by Castell and MacLean (1964).

In vivo protection against peroxides is well established as a dietary need of animals (Dam, 1964) including fish (Woodall et al., 1964). Witting (1965) has discussed the potential relationship between antioxidants (such as α-tocopherol) and the number of double bonds in fatty acids of the body depot and cellular fat of animals. Antioxidants are necessary both to offset peroxides in dietary fat and as more basic cellular materials necessary to neutralize peroxides inevitably formed in body processes.

The catabolism of body fat does not apparently require much α-tocopherol in the cod, as all three starved fish, including the nearly moribund fish H, retained about 50% of the tocopherol estimated as normal for fish of comparable size. The livers of fish I and J were nearly normal in appearance, but that of fish H was notably dark-brown in colour (possibly due to ceroid, cf. Dam, 1964; Woodall et al., 1964). The distribution of α-tocopherol in the flesh and liver of fish H was surprising, but the estimated concentrations of α-tocopherol in the livers were inversely proportional to the lipid contents for the three fish (Table II). In several fish species normal or seasonally lean livers are found to have high α-tocopherol levels (e.g. 700, 1540, 3100, 7000 μg/g lipid; Braekkan et al., 1963) but the α-tocopherol level in proportion to lipid in the liver of fish H is clearly abnormal for cod. The high α-tocopherol level in the flesh probably followed displacement from the liver as a result of failure of liver organization or function.

The flesh lipid content of fish I and J (0.76%) was typical of cod in general (Bligh and Dyer, 1959; Bligh and Scott, 1966; cf. Table I), and the liver weights and fat contents indicated that these fish were in no worse condition than

many cod in the natural state in periods of food scarcity (Jangaard et al., 1967). Fish H corresponded in all respects to the drastically starved cod described by Love (1958), where it was found that the livers were red in colour and the lowest possible liver lipid level was about 2%. Love observed that the brain fat content was not affected by starvation and that muscle tissue intercellular space did not increase (cf. jellied plaice, Templeman and Andrews, 1956). The muscle lipid was not determined by Love, but the water content of the muscle rose from a normal 80–81% to 84–88%. From these figures non-lipid solids in muscle tissue may be taken as 20% for normal cod and 14% for starved cod. Proportionating the 0.76% muscle lipid typical of healthy cod gives a figure of 0.53% lipid for a drastically starved cod and in the present study (Table II) the flesh of fish H was in fact found to contain 0.54% lipid. This emphasizes the strictly cellular nature of cod flesh lipids suggested by the types of lipid found by Bligh and Scott (1966).

Despite the possible liver damage in fish H the large amount of α-tocopherol in the fish as a whole suggests that the pathological condition of the fish was not associated with any deficiency in α-tocopherol.

Dogfish

The dogfish samples were of two different origins, although dorsal flesh was taken in both instances. Fish A was handlined off Eastern Passage, N.S., in July, and analysed after 1 day in the live-holding tanks. Fish B was taken on the La Have Bank, by trawl, in February and held on ice for several days before analysis. No comparable data are available for North Atlantic Squalidae but an Indian ocean shark (*Carcharias ellioti* Day) has a flesh content of about 30 μg/g α-tocopherol (Nazir and Magar, 1964) similar to fish A. Dogfish are migratory (Templeman, 1963), but there is inadequate evidence to associate the low α-tocopherol value in fish B with migration, diet, gonad development, or handling of the fish after death.

Flounder

The flounder results are based on commercial "sole" fillets obtained shortly after freezing at a commercial plant. The fish had been iced for 2–4 days after catching. The results of the freshly frozen samples are perhaps comparable to those obtained by Braekkan et al. (1963) for halibut, estimated as 178 μg/g lipid. The significance of these samples, which will be published in detail elsewhere, lies in the observation that under excellent commercial storage conditions the α-tocopherol content of the flesh for most samples fell below detectable limits at some point in time between 4 and 8 months. Ikeda and Taguchi (1966) found that α-tocopherol declined in muscle and visceral organs of yellow tail mackerel (*Seriola quinqueradiata*) in the course of frozen storage.

Mackerel

The mackerel was taken locally in June as part of the northerly migration of sexually maturing fish past Nova Scotia. Unfortunately, the sex of the partic-

ular fish was not recorded. Jensen and Kringstad (1942) reported 16–18 μg/g α-tocopherol in mackerel muscle, and the present value is also similar to the 16 and 14 μg/g in herring muscle noted by Braekkan et al. (1963). The flesh lipids of these ripe herring were respectively 14 and 15.5% in males and females. Sexual maturation in herring evidently affects the liver α-tocopherol in particular, as Braekkan et al. (1963) observed 115 μg/g tissue in male livers but only 14 μg/g tissue in female livers. Perhaps correspondingly the ovaries were richer in α-tocopherol than the testes.

Sablefish

This Pacific species was examined since the oil was reported to be resistant to oxidation and to contain a high level (628 μg/g) of α-tocopherol (Einset et al., 1957). Examination of another sample by Dolev and Olcott (1965) indicated only 224 μg/g oil. The respective iodine values of these two oils were 99 and 80 (the latter on the isolated triglycerides). Dolev and Olcott (1965) recovered 9.8% oil from the muscle tissue. In the fish examined in the present study (commercial catch, British Columbia, October, iced for an unknown period before freezing and shipment for study) the lipid recovered by the method of Bligh and Dyer (1959a) was 15.9% with an iodine value (Wijs) of 82. The α-tocopherol content of 270 μg/g lipid (chiefly triglycerides), and the iodine value, are comparable with those in the fish analysed by Dolev and Olcott (1965). The two literature sources stress the resistance to oxidation of whole oil, and Dolev and Olcott (1965) mention phospholipids in whole lipid extracts as potential antioxidants, although these materials apparently have only a synergistic effect (Olcott and Van der Veen, 1963). It is possible to ascribe the resistance to oxidation demonstrated for one lot of sablefish oil (Einset et al., 1957) solely to a high α-tocopherol content, and similarly the resistance to oxidation of a high iodine value (192) herring oil from small spring herring (Astrup, 1964) may have been due to a high α-tocopherol content. The small size of the herring and high iodine value of the oil in the latter report suggest that these fish may have been feeding heavily on phytoplankton, as it is known that the fatty acids of the lipids of several species of phytoplankton have iodine values of the order of 200 (Ackman and Tocher, unpublished observations). The resistance to oxidation may therefore have been due to high tocopherol levels also originating in the phytoplankton (Ackman and Cormier, unpublished observations). However, in the sablefish oils examined, two out of three analyses place the α-tocopherol level in the range normal for the lipids of the flesh of marine fish (Table I).

The concept of a low iodine value for marine lipid fatty acids as an index of potential resistance to oxidation can be misleading. Monoethylenic fatty acids markedly resist oxidation in comparison with the notoriously susceptible polyunsaturated fatty acids (Privett and Blank, 1962), but the low iodine value sablefish oil examined by Dolev and Olcott (1965) had a total of 13.2% polyunsaturated fatty acids. This figure is not far below the polyunsaturated fatty acid content of many marine oils not noted for resistance to oxidation

(Ackman, 1966). In this respect it is interesting that Roach et al. (1966) observed that the peroxide values for the lipids from red lateral muscle were the same for sablefish and lemon sole after 14 days on ice. The sole would be expected to have both a high proportion of phospholipids and of polyunsaturated fatty acids in comparison with the sablefish (Olley and Duncan, 1965). There does not appear to be any particular reason for ascribing the generally reported resistance to oxidation of sablefish oil to any one factor.

Lobster

The lobster results have been included in the fish data because of the comparable omnivorous diet of this crustacean. The two lobsters A and B were obtained from Eastern Passage, N.S., in June and held in live-holding facilities for 4 months on a diet of beef liver and whole sardine herrings. The condition and size of the livers at killing were interpreted as indicating a healthy condition, and lobster A was in a late pre-moulting state. The fat determinations of Bligh et al. (1957), based on cooked meats, showed that the tips of the claws in lobster might contain 1.6% fat, but for the bulk of the meat the figure was about 1 ± 0.1%. In crabs the lipid content is also of the same order (Thompson, 1964). The only comparable α-tocopherol data is that reported by Braekkan et al. (1963) for a lipid extract of the deepsea prawn, *Pandalus borealis*, which had a α-tocopherol level of 950 μg/g lipid (estimated at 28.5 μg/g fresh weight). The lobster liver α-tocopherol contents are 30–50% of those quoted for most fish livers by the same authors. If it is assumed that most of the α-tocopherol in the prawn is located in the flesh, the agreement with the high figure for lobster meat is not unreasonable. A possible explanation for the high α-tocopherol level in lobster flesh is the associated presence of oxidation susceptible pigments (Bligh et al., 1957b).

Lobster C was obtained from Eastern Passage, N.S., in April, and held for 5 days before it was killed. The flesh result was higher than those for lobsters A and B but not sufficiently different to indicate that the low levels for A and B were due to prolonged live holding. The liver of lobster C was inadvertently lost.

Oyster

The oyster results on single fresh specimens suggest that the amount of α-tocopherol is larger in the larger specimens (see also mussel results, Braekkan et al., 1963). Too few specimens were examined to establish this as more than a suggestion at this time. Thompson (1964) found 2.0–2.4% lipid in this species of oysters. The oysters live-held for extended periods developed a fetid odour (see also periwinkles) and the adductor muscles became progressively weaker. The fall in lipid content was at first paralleled by the decrease in α-tocopherol, but at some point after 4 weeks the α-tocopherol became undetectable.

Bay Quahog

The α-tocopherol contents of the whole animal were of the same order as for the oyster, but owing to the very low lipid contents in comparison with

the other freshly collected shellfish the values per gram lipid were exceptionally high in quahogs live-held 1 and 2 weeks. If the absence of α-tocopherol after only 4 weeks is considered, the original fresh values may well have been higher. There is no definite explanation for the low lipid recovery (note that the closely related ocean quahog also has a low lipid content when compared with the other shellfish). If this is a cellular level lipid (compare cod H, and live-held oysters), the rapid elimination or utilization of α-tocopherol could be explained as there would be none in depot fat reserves (cf. starved cod results). Gras (1963) and Bourcart et al. (1964) have shown that in the European mussel (*Mytilus galloprovincialis* Lmk.) the seasonal fluctuations in lipid (0.8–3.2%) are reflected proportionately in both total lipids and phospholipids, and that adipo-granular cells diminish from the beginning of the sexual cycle. Shimma and Taguchi (1964) studied a number of shellfish with a somewhat unusual extraction procedure which may have resulted in some loss of phospholipids. Their results give 0.34–0.82% lipid for body muscle (?) tissue, 0.77 and 0.89% for two species where the viscera were included, and 3.4% for a whole oyster (*Crassostrea gigas*). Thompson (1964) found 1.6% lipid in scallop (*Placlopecten megallanicus*) adductor muscle. Gruger et al. (1964) report lipid contents of 2.1%, 0.5%, 2.5%, and ±1% respectively for canned meat of blue crab (*Callinectes sapidus*), whole littleneck clam (*Protothaca staminea*), whole Pacific oyster (*Crassostrea gigas*), and scallop muscle. Another possible explanation of the rapid decline in α-tocopherol content of the quahogs would be that the larger part of the α-tocopherol originally present is derived from plant cells passing through the gut as suggested by Fisher et al. (1964) for β-carotene observed in certain crustacea. Unfortunately, this view opens an entire new field for speculation with little reliable data available. However, it is known that oysters and other molluscs can accumulate dimethyl-β-propiothetin, apparently in the viscera, and eliminate the thetin slowly over long periods of live holding (Ackman, unpublished observations; see also Ackman et al., 1966b). The presence of the thetin may be due to deposition in the animal tissue or accumulation in the digestive tract of undigested algae or parts of algae rich in thetin (Ackman et al., 1966a).

Ocean Quahog

This species tends to live in deeper waters (>50 ft) than most of the other shellfish examined. Under these conditions the dietary intake of live phytoplankton would probably be less than in the oyster and bay guahog, and the α-tocopherol content accordingly lower.

Mussels

Little can be said of the horse and ribbed mussel results except that these species have about the same content of α-tocopherol on any basis as the fresh oyster with the same lipid content, and as the blue mussel from the same area, also of similar lipid content.

The blue mussels from Halifax harbour are more noteworthy. Those from the bottom of the harbour were obtained from seawater intake lines at a depth

of 50–60 ft. It is considered that this position in the harbour is primarily in a current of oceanic water entering the harbour. The α-tocopherol contents are similar to those of the mussels from a normal environment (Smelt Creek), presumably reflecting a reasonable plankton intake. The shoreline mussels were taken in the intertidal zone only a short distance from a major sewage outfall. The proportion of organic detritus in the diet would therefore likely be higher with a corresponding lower level of α-tocopherol. The food of filter feeding organisms is, however, always a controversial question (Jørgensen, 1962; Parsons, 1963).

Periwinkle and Dogwinkle

The high α-tocopherol level of the periwinkles was not unexpected as Braekkan et al. (1963) had reported 40–45 μg/g fresh weight, and ascribed this high level in herbivorous littoral snails to their feeding directly on algae with no detritus in the diet. On live-holding periwinkles the results were similar to those observed with the oyster. After 2 weeks the animals were very weak, developed an offensive odour, and lost both lipid and α-tocopherol.

The dogwinkle, like the whelk (*Buccinum undatum*) examined by Breakkan et al. (1963), is a predatory snail, eating other molluscs. These particular dogwinkles were collected with the Terence Bay periwinkles, and the α-tocopherol content may have been fairly high due to this association (the whelk value was 8 μg/g fresh weight).

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Behaviour and Ecology of Sockeye Salmon Fry in the Babine River[1]

By P. McCart

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

ABSTRACT

In the Upper and Lower Babine rivers the fry of sockeye salmon (*Oncorhynchus nerka*) emerge and move downstream, predominately at night, before reaching low-velocity areas along the stream margins. After a period of days or weeks these fry migrate upstream, close inshore, during the day. The diel pattern of upstream movement appears to be bimodal. The seasonal periodicities of both downstream and upstream movement are related to water temperatures.

Upstream migrants were larger than downstream migrants in the Upper Babine River but not in the Lower Babine River. Upper River upstream migrants were similar in length to Lower River upstream migrants during 1964 but not in 1965. In both years Upper River fry weighed proportionately more than Lower River fry, and in both rivers 1964 fry weighed proportionately less than 1965 fry. Some possible explanations of these observations are discussed.

Early in the season, fry tend to disperse alongshore after entering the lake. Later they leave these inshore areas and become entirely pelagic.

The principal foods of fry both in the river and in the lake were copepod and cladoceran plankters.

Both fish and birds were found to be preying on sockeye salmon fry. Fry appear to be more vulnerable to predation in the river than in the lake.

The upstream movement of fry in the Babine River does not appear to differ appreciably from upstream movements in other areas. A comparison of fry movements in the Babine River and the Fulton River, where fry move only downstream, suggests that the differences in behaviour are genetically rather than environmentally induced.

It is suggested that, because of high mortalities in the prepelagic period, upstream-migrant populations will only develop where the environment of both the river and the rearing lake are especially favourable.

INTRODUCTION

The young of anadromous sockeye salmon, *Oncorhynchus nerka*, typically spend 1 or more years in the fresh waters of lakes before migrating to the ocean. Returning adults spawn either in lakes or in streams. Most commonly, the latter are streams flowing into the nursery lake and, upon emergence, fry move immediately downstream. In some instances, however, the parent populations spawn in the lake outlet and the young move upstream. There is a considerable body of information available describing the behaviour of sockeye fry moving downstream but, as yet, no detailed description of an upstream migration.

In two of the numerous streams utilized by spawning sockeye salmon at Babine Lake, British Columbia, large upstream fry migrations are an annual

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occurrence. During the spring and early summer of both 1964 and 1965 a study was made of the behaviour and ecology of sockeye fry in these streams, the Upper and Lower Babine rivers. Some comparisons were made with sockeye fry in the Fulton River, a Babine Lake tributary in which fry move downstream.

THE AREA OF INVESTIGATION

The accompanying map (Fig. 1) illustrates features of the Babine Lake area pertinent to this study.

The spawning grounds of the Upper and Lower Babine rivers and the Fulton River are the most heavily utilized in the Babine Lake area, receiving 23.4%, 16.9%, and 15.6% respectively of an average annual escapement of 486,000 sockeye spawners.

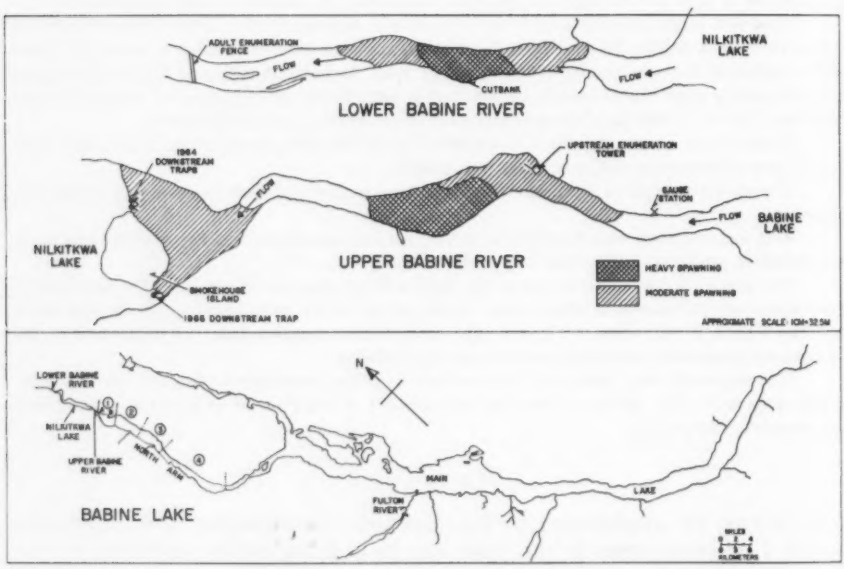

FIG. 1. The Upper and Lower Babine River spawning areas and Babine Lake.

The Upper Babine River is about 2296 m (7000 ft) long and averages 74 m (225 ft) in width. Emergent fry can enter nursery areas either downstream in Nilkitkwa Lake or upstream in the North Arm of Babine Lake. Quiet areas and grassy sloughs are common at the river outfall and along the banks, especially on the west side. Water velocities are low, generally less than 30 cm/sec (1 ft/sec). Several small streams enter the Upper Babine River but these have little effect on conditions in the river. Water temperatures in the main flow are generally lower than those at the lake surface and ap-

proximate the temperatures found between 2 and 5 m (Fig. 2). Water entering the river is probably a mixture of near-surface and deeper, colder waters. Water temperatures in the river are subject to large day-to-day fluctuations, presumably the result of wind-influenced changes in the thermal structure of the North Arm of Babine Lake.

The Lower Babine River is similar in width to the Upper Babine River but water velocities are much higher, approximately 122 cm/sec (4 ft/sec) in the main stream and up to 244 cm/sec (8 ft/sec) through the weir. There are few extensive areas of slow water along the banks to serve as refuges for holding fry and upstream migrants. Two substantial streams enter the Lower

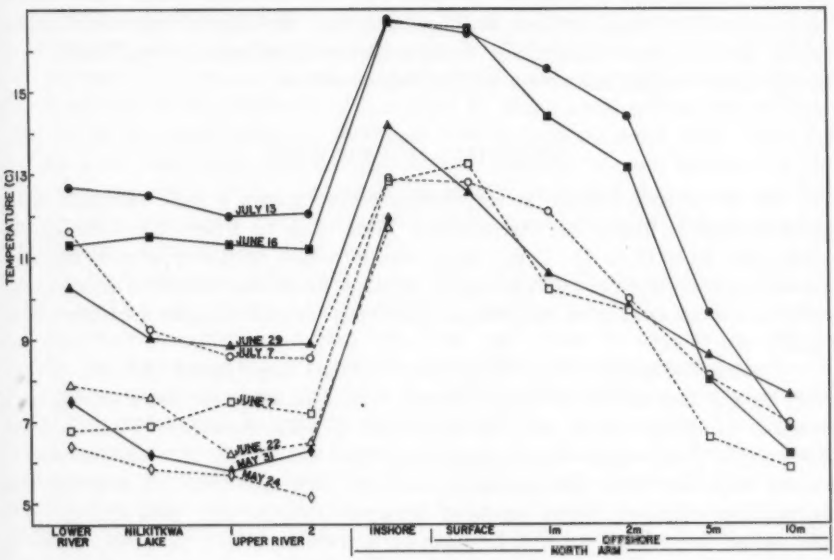

FIG. 2. Water temperatures in the North Arm, Upper and Lower Babine rivers, and Nilkitkwa Lake at various times during 1965.

River just below Nilkitkwa Lake, one on the east and one on the west. These are frequently in flood during the period of fry migration and can markedly affect the turbidity of the main stream.

Sockeye spawning in the Lower Babine River is almost entirely confined to the length of river between the enumeration weir, or fence, and the lower end of Nilkitkwa Lake, a distance of approximately 1640 m (5000 ft).

Salmon spawn over a considerable length of the Fulton River, 3281 m (10,000 ft), between Babine Lake and an impassable 13 m (40 ft) falls below Fulton Lake. The river averages 25 m (75 ft) in width and water velocities at the time of fry emergence are relatively high. Emerging fry move down-

stream into the main basin of Babine Lake. The river becomes turbid during extreme high water but is reasonably clear during a large part of the downstream fry run.

Fry moving upstream in the Upper Babine River eventually enter the North Arm of Babine Lake. For purposes of this study the North Arm was divided into four major sampling areas. Lake area 1, closest to the river, was further divided into subareas 1A and 1B. The lake shelves off rather rapidly throughout the area of investigation and most of it exceeds 10 m in depth. Areas of shallow littoral and emergent vegetation have a patchy distribution, generally in small bays and alongside the alluvial fans of the few, small tributary streams. Two of these streams support small sockeye spawning populations, a long-term average of less than 1400 fish between them. Movements of fry from the main body of Babine Lake into the North Arm are thought to be small so that almost all the underyearling sockeye in the North Arm are progeny of fish spawning in the Babine River.

MATERIALS AND METHODS

In the Upper Babine River, downstream migrants were sampled with traps located in relatively fast-moving water close to where the river enters Nilkitkwa Lake (Fig. 1). These traps, modifications of the type used by McDonald (1960), were constructed of 3.15 mesh/linear cm (8 mesh/linear inch) hardware cloth over plywood frames. The trap mouths measured 61 cm (2 ft) × 122 cm (4 ft).

During 1964, from May 15 to July 17, two traps fished the arm of the river to the east of Smokehouse Island. The traps were tended morning and evening at about 0800 and 2000 hr PST (Pacific Standard Time is used throughout this paper). Catches were recorded separately as *nocturnal downstream migrants*, those fish captured between 2000 and 0800 hr, and *daytime downstream migrants*, those captured between 0800 hr and 2000 hr. In fact, the period from 2000 to 0800 hr includes a number of daylight hours and a proportion of the fry classified as nocturnal migrants must have entered the traps during daylight.

Two or three times a week, hourly counts were made between 2000 and 0500 hr to determine the periodicity of nocturnal downstream movement. These counts were corrected to compensate for the period of time necessary for the removal of fry from the trap, generally about 10 min of each hour.

During 1965, a single trap was fished from May through July in the west arm of the river. As in 1964, daily counts were made at 0800 and 2000 hr. No hourly counts were made.

In both years, counts of fry moving upstream along the east bank of the Upper Babine River were obtained by estimating the number of fish passing over a 1.8 m (6 ft) square, white grid placed on the stream bottom. Fish were guided across the grid by leads constructed of hardware cloth and were viewed by an observer on a tower placed directly over the grid. Small

numbers of fry were counted directly but, during periods of high activity estimates were made by groupings of 10, 100, or occasionally 1000.

On 7 days in 1964, records were kept of the speed at which fry moved upstream across the counting grid. These speeds were corrected by the addition of the speed of the water flow across the grid to give the true speed of the fish through the water. For each date the speeds of several hundred individual schools were averaged. The swimming speed of fry may have been affected somewhat by the presence of the grid but there was no apparent change in their behaviour or speed of movement.

During 1964, counts of upstream migrants were continuous throughout an 18- to 20-hr period, generally from 0200 to 2200 hr. In 1965 the daily counts were for 4 hr only, 1600 to 2000 hr. The 4-hr index was adopted to obtain a better measure of seasonal variation in upstream movements than was provided by the infrequent 20-hr counts made in 1964. In 1964, an average 27.5% of the daily run passed upstream during these 4 hr, ranging from a low of 3.4% on July 14 to a high of 65.2% on July 17. Figure 3 illustrates the relationship of counts made between 1600 and 2000 hr and the total daily count for 22 days in 1964. This relationship, though variable, is close enough for the purposes for which it was intended, the identification of extremes. In no case was a very high total count associated with a low 4-hr count nor a very low total count with a high 4-hr count.

In both years, samples of fry were taken in the Upper and Lower Babine rivers and in the North Arm of Babine Lake for length-frequency studies. Downstream samples were obtained from the traps in the Upper Babine River; in the Lower River (1964 only), with dip nets lowered into the water at various positions along the adult counting fence. Upstream migrants were captured in the area called the "cutbank" in the Lower Babine River and in the area close to the upstream enumeration tower in the Upper River. These were occasionally dipnetted but in most instances a small-mesh, nylon marquisette seine was used. The same seine was used to capture fry along shore in the North Arm of Babine Lake. In 1965, a few samples of fry were also taken offshore in a 2.74 m (9 ft) square townet similar to that used by the Fisheries Research Institute of the University of Washington in Alaskan lakes. All townetting was done at the water surface at dusk and shortly thereafter.

All lengths and weights were obtained from live fish anaesthetized in a 0.02% solution of 2-phenoxyethanol. Individual *standard lengths* were recorded as pin pricks on millimeter graph paper. Fry were blotted with paper towelling to remove excess moisture and then weighed in air on a Mettler K 7 balance. The weight was recorded adjacent to the appropriate pin prick. These techniques, a modification of those described by Parker (1963), were identical in both years.

Many of the fry emerging from the gravel in the Upper Babine River still retained a portion of the larval yolk supply. Anticipating that there might be differences in the behaviour and ecology of fry with and without

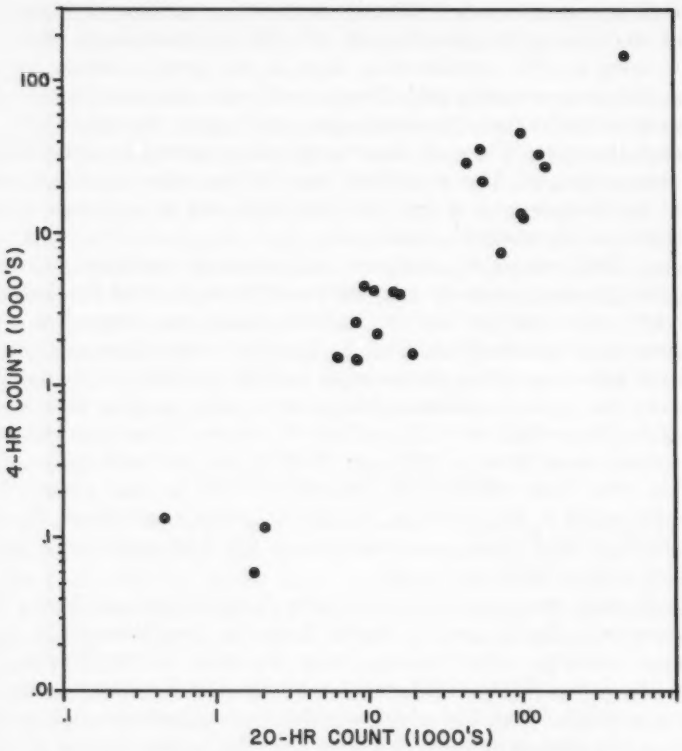

FIG. 3. The relationship of counts made between 1600 and 2000 hr to the total daily upstream count (20 hr). Data for the Upper Babine River, 1964.

yolk, records were kept of the presence or absence of an external yolk sac in fry from samples taken for various purposes. In most instances, fry with yolk sacs were further classified as having either large yolk sacs (large enough to be seen laterally) or small yolk sacs (a slit visible from the ventral surface only). Some fry without externally visible yolk sacs were examined after preservation to determine whether they still contained vestiges of yolk internally.

During 1965, samples of fry were taken for food studies at approximately weekly intervals at a number of localities in the Upper and Lower Babine rivers and in the North Arm of Babine Lake. On two occasions, fry were captured at 2-hr intervals, over a total period of 24 hr, along the shores of Smokehouse Island where the Upper River enters Nilkitkwa Lake. The numbers of food organisms were counted directly or, where very large numbers of organisms were involved, estimated with aid of a counting grid. In addition

to counts of individual organisms, visual estimates were made of the percentage of the total food volume falling into each of the following groups: unidentified insects, unidentified plankters, all insects, and all plankters.

Plankton samples were taken on the same or the following day for comparison with the stomach contents of fry. These were taken using Clarke–Bumpus samplers with No. 10 standard nets. Water temperatures were taken at the same time using an electronic thermistor. The methods of counting plankters were the same as those used in stomach analysis.

Samples of predatory fishes were taken either in gill nets or in a large beach seine. The monofilament nylon gillnets were assembled in standard gangs with six 7.6 m (25 ft) × 2.6 m (8 ft) panels of the following sizes: 2.5, 3.8, 5.1, 6.3, 7.6, and 10.2 cm (1, 1½, 2, 2½, 3, and 4 inch) stretched mesh. The seine, 39.3 m (120 ft) long and 2.6 m (8 ft) deep, varied from 5.1 cm (2 inch) stretched mesh on the wings to 2.5 cm (1 inch) in the bag. Predators were weighed, measured, and sexed, and scale and stomach samples were taken. The stomachs, preserved in 10% formalin, were examined later under a binocular microscope.

In both years, water levels were recorded daily at a gauging station established by the Department of Energy, Mines and Resources on the Upper Babine River. A thermograph nearby kept a continuous record of stream temperatures. On occasion, changes in light intensity were followed with a Photovolt 200 photometer recording directly in foot candles.

Howard Smith of the Fisheries Research Board, working on the Lower Babine River, provided valuable data on environmental conditions and the movements of fry in the Lower Babine River. The Resource Development Branch of the Department of Fisheries of Canada provided similar information for the Fulton River.

GENERAL DESCRIPTION OF BEHAVIOUR

During both years of the study, frequent observations were made of the distribution and behaviour of sockeye fry in the Babine Rivers. These observations, largely qualitative, are presented here to acquaint the reader with the general pattern of fry movement before the more detailed aspects of the study are discussed.

The activities of fry in the Babine River can be conveniently divided into three phases: downstream movement, holding, and upstream movement.

Behaviour of Fry Moving Downstream

Large numbers of fry moved downstream in both the Upper and Lower Babine rivers. This movement was predominantly nocturnal but some daytime movement did occur. The behaviour of fry moving downstream after dark was not observed but, on several occasions, downstream migrants were seen during the day from a boat anchored in midstream in the Upper Babine

River. Of 76 fry seen on June 29 and July 2, 1965, all but 10 were single individuals. There was one group of 4 scattered about 30 cm apart and 3 pairs with 30–60 cm separating individuals. Seventy-two fry were at the surface; 4 others at depths of 15–30 cm. Of the 76, 33 were swimming actively downstream, 8 were drifting down tail first, 33 were heading obliquely across the current, and 2 were swimming upstream. All these fry were small and some had visible yolk sacs. Such behaviour was observed on a number of other occasions. The behaviour of daytime downstream migrants was similar to that of the nocturnal migrants described by Hartman et al. (1962) which "migrated as individuals, facing downstream, and usually exhibited swimming movements." A significant departure was the frequent occurrence of fry which, though moving downstream, were swimming at a considerable angle to the main current. This tendency may be an important mechanism enabling fry to reach the edges of the river before being swept very far downstream.

J. Roos (personal communication) of the International Pacific Salmon Fisheries Commission, New Westminster, B.C., reports an interesting experiment which emphasizes the ability of sockeye fry to reach the stream margins. He released a number of dyed upstream migrants at night in the middle of the Chilko River. The following day some of these fry were recaptured moving upstream, alongshore, at a point opposite the point of release suggesting that the fry had not moved very far downstream before reaching the river's edge. These fry were not newly emerged but the ability of recent emergents to reach the river's edge may not be at all inferior. In the broad, fast-moving Lower Babine River, millions of fry reach the banks before they have passed downstream as much as a mile.

Another possibility was entertained, that fry reach the edges of the stream by swimming along the stream bottom, but not a single fry was located offshore along the bottom in the course of an underwater (scuba) search in the Upper Babine River. At the moment, the behaviour of fry at first emergence and the manner in which they reach the stream edge is still very much a matter of conjecture.

Behaviour of Holding Fry

After reaching the river margin, fry may pass several weeks before completing their journey upstream. These fry accumulate in backeddies, sloughs, and other areas of quiet water along the river's edge. In the Upper River thousands more gather in areas bordered by brush and emergent vegetation around Smokehouse Island and the river outfall. These "holding" fry were observed on many occasions. There is also some indication that many Upper River upstream migrants have lived for a time in Nilkitkwa Lake before entering the river and moving upstream.

Early in the season, schools of holding fry were small and scattered (a maximum of about 200 fry) and solitary individuals were common. Later, groups of several hundred thousand fry often accumulated in favoured holding areas.

During the day, undisturbed fry swam near the water surface. Schools were loosely organized and there was a great deal of individual activity, generally associated with feeding. The orientation of schools was basically rheotactic but where currents were slight there was much wandering about.

The behaviour of sockeye fry in response to disturbances, either by observers or predators, appeared to change after schooling. Some of the single individuals observed holding along the stream edges very early in the season buried themselves in the bottom material when disturbed. Fry in groups were never seen to do this. When mildly disturbed they moved as a group, away from the disturbance, often sounding, and in among brush and vegetation alongshore. When violently agitated they scattered wildly; however, schools soon formed again.

At night, schools of fry in fast water generally remained close to the bottom but were frequently observed near the surface in quieter areas. Observations of single schools indicated that the majority of these remained in the same area throughout the night even at temperatures as low as 5 C. This contrasts with the behaviour of schooling sockeye fry described by Hartman et al. (1962) in Hidden Creek at Brooks Lake, Alaska. In this stream, holding areas were completely evacuated during the nightly outmigration and filled again at dawn.

The holding period, during which fry are concentrated in a narrow strip along the edges of the stream, is undoubtedly one of high mortality. Hartman et al. (1962) have discussed the survival value of protected, inshore holding areas as refuges from piscivorous predation. Observations on the Upper Babine River suggest that there are additional advantages to fry inhabiting these areas for extended periods of time. Currents immediately alongshore are slow, often negligible, and, as Fig. 4 shows, water temperatures are comparatively high. These conditions were most exaggerated in some of the small sloughs along the west bank of the Upper River. On June 3, 1965, a temperature of 14.8 C was recorded in a flooded grassy area where there were many fry. The temperature of the main stream 6 m away was 5.3 C. Together, higher water temperatures and slower currents probably encourage rapid growth.

Behaviour of Fry Moving Upstream

Sockeye fry moving upstream did so almost entirely in daylight. The movement was confined to the stream edges. The first, early morning migrants were individuals or, more rarely, small groups of up to 10 fish swimming in a characteristic manner: a series of short, quick spurts followed by periods of holding or of downstream movement during which fry drifted tail-first. The fish appeared to have difficulty in maintaining position and the movements of fry in groups were not well synchronized. As light intensities increased, so did the size of groups and hesitant, individual, swimming movements were replaced by highly organized schooling behaviour.

Where they were unhindered, migrants moved in tightly knit schools at the surface close to shore, often in water only a few centimeters deep. The

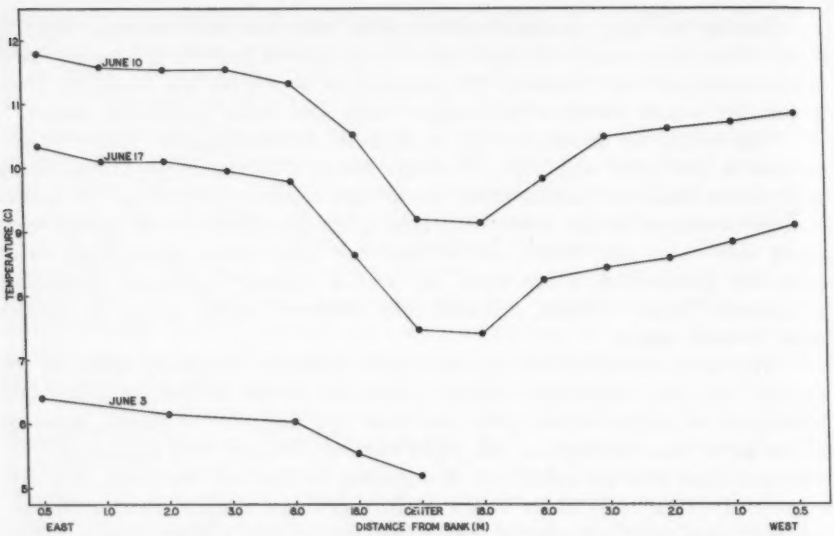

FIG. 4. Mean water temperature across the Upper Babine River on three occasions in 1965.

average school numbered from 100 to 200 individuals but many were larger. On June 26, 1964, an almost continuous band of fry moved across the counting grid for a period of 26 minutes.

The average speed of schools on the 7 days when records were kept ranged from 18.3 to 32.1 cm/sec with a mean of 24.9 cm/sec. The average speeds for 6 of the 7 days exceed the mean maximum sustainable cruising speed of from 15.3 to 20.1 cm/sec given by Brett et al. (1958) for fish of similar size (30 mm) in water of similar temperatures (6–10 C) but are comparable to the average 24.4 cm/sec recorded by Andrew and Geen (1960) for Chilko River upstream migrants. Possibly Brett's laboratory-reared fry had less stamina than natural fry. Another possibility is that upstream migrants are able to maintain a speed in excess of their maximum sustainable swimming speed by alternating periods of rapid upstream movement with periods of holding and resting.

Fry usually swam around rather than under floating objects. After a period of hesitation during which the school moved back and forth searching for alternate routes, the fry either rounded the obstruction at good speed and continued upstream (after moving back into shallow water) or, occasionally, retreated downstream.

In some instances (especially in the Lower River) fry encountered short stretches of extremely fast water. Here, fry spread out over the bottom close behind, and frequently in contact with, protruding stones and vegetation, apparently taking advantage of small eddies and areas of turbulence. Individuals and small groups moved upstream from holding point to holding

point in a series of short, quick spurts. In one particularly fast section of the Lower River, fry were observed moving along the bottom as much as 5 m offshore, at a depth of 1.5-2.0 m.

Current direction was an important factor in the orientation of migrants while they were still in the river. In some situations where current patterns were confused in the immediate inshore area (as they were in large backeddies) actively migrating fry moved upstream along the current interface even when this took them 10 m or more offshore over deep water. Similarly, fry moving past flooded grassy verges or the entrances to sloughs usually remained along the edges of the main current rather than taking advantage of the adjacent areas of dead water. They avoided the areas frequented by holding fry. Current responses of this sort would eventually bring fry up into the lake. Occasionally, however, fry were observed moving alongshore, into and through large backeddies, with no apparent hesitation even though the direction of the current changed through 180°. This, and the fact that, especially early in the season, many schools maintained their southward orientation for many miles up the lake, suggest that there may be other important orientational cues.

As light intensities fell toward evening, the behavioural changes observed during the morning were reversed. Fish moved in smaller and smaller units and upstream movement became erratic. Loosely knit groups of fry accumulated in suitable holding areas alongshore and mass movement of fry ceased. That fry were then unresponsive to visual stimuli is indicated by the fact that they did not react to a hand waved repeatedly only 30–40 cm above them. In the Upper Babine River few fish were seen to lose position in the dark. Most of the groups holding either on the grid or upstream from it appeared to maintain their integrity throughout the night although on occasion fry were observed fleeing downstream under attack by predators.

During May and June 1965, many of the schools of fry entering the North Arm of Babine Lake continued to move southward close inshore. Fry were seen inshore, moving southward, as far uplake as Halifax Landing, a straight-line distance of approximately 32.2 km. During July, however, it appeared that only a small proportion of the fry entering the lake behaved in this way. The numbers of fry seen inshore declined to almost zero within 400 m of the river and a number of large schools were observed swimming directly out into the lake a few hundred meters beyond the river.

SEASONAL PERIODICITY OF FRY MOVEMENTS

Both the upstream and downstream movements of fry in the Upper Babine River were characterized by a succession of peaks or surges (Fig. 5). In 1964, downstream movement was underway when trapping began in mid-May. In 1965, the first captures were made on the second night of trapping. In both years there was a gradual increase in nocturnal downstream catches up to mid-June and then two prominent peaks. Catches declined thereafter

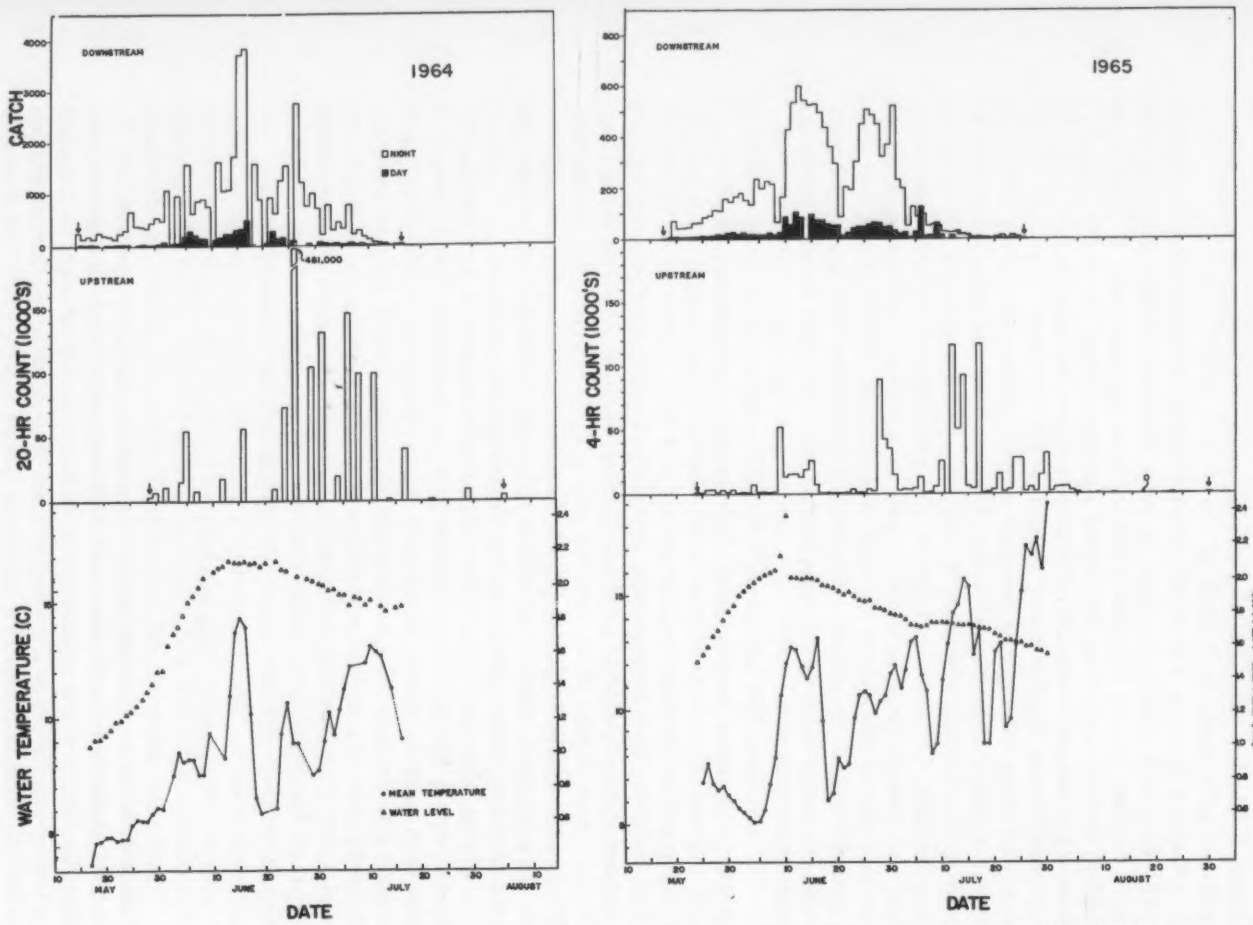

FIG. 5. Major trends in the seasonal periodicity of sockeye fry movements in the Upper Babine River in 1964 and 1965. Data are recorded separately for nocturnal downstream migrants (those captured between 2000 and 0800 hr), daytime downstream migrants (0800–2000 hr), and upstream migrants and are shown along with the seasonal fluctuations in water level and water temperature. Arrows indicate the dates between

and nocturnal movement was essentially finished by mid-July. This long period of downstream activity, of presumably newly emerged fry, probably reflects the long spawning period of the parent fish, from late September to December.

The daytime downstream catch constituted an important segment of the total (9.0% in 1964 and 13.7% in 1965), even exceeding the nocturnal catch on a number of occasions during the latter part of the 1965 season. Its actual importance may be even greater than the figures indicate in that trap avoidance probably affected catches more during daylight than in the dark and because an unknown number of fry which had actually moved downstream in the early morning daylight were included with the nocturnal segment of the downstream run.

Although the 1964 data are incomplete, there was, in both years, a general correspondence between the peaks of daytime and nocturnal trap catches. Several obvious exceptions are the relatively large daytime catches made subsequent to July 5, 1965. These catches may reflect an increased tendency to daytime emergence during the latter part of the season. The fact there is little correspondence between the numbers of fish moving downstream during the day and the counts of upstream migrants suggests that the former were not simply fry displaced while moving upstream. On several occasions in 1964, daytime and nocturnal downstream migrants taken on the same day were compared. There was no apparent difference in either the degree of development of the fry or their length-frequencies.

The only comparable rate of daytime downstream movement in sockeye fry under stable natural conditions is that recorded by Heard (1965) for the Brooks River in Alaska, 11.0%. Heard (1965) suggests that these large daytime catches resulted from the fact that the traps were located on or immediately adjacent to gravels from which fry were emerging and that these were caught while making short excursions out of the gravel during daylight. The traps in the Upper Babine River were located close to the spawning gravels. However, daytime downstream migrants were readily observable in this river and their behaviour, already described, did not appear to support Heard's suggestion. On occasion, the paths of individual fry could be followed for 30 to 40 m. The fry were at the water surface, sometimes over considerable depths, and were not seen to make any attempt to hide or rebury themselves. Some were seen swimming with the current on into Nilkitkwa Lake.

The 1964 upstream data are incomplete. The tower counts, together with visual surveys of the stream edge, indicated that few fry were moving upstream when counts began in late May and early June; that fry were most abundant and active from about June 20 to July 15; and that few fry were present in the river from late July on into August. This would suggest that most of the upstream movement took place when downstream movements were already in decline.

Except for one day (July 11), the 1965 upstream data are complete from May 24 to August 5. There were three distinct surges in upstream movement,

FIG. 5. Major trends in the seasonal periodicity of sockeye fry movements in the Upper Babine River in 1964 and 1965. Data are recorded separately for nocturnal downstream migrants (those captured between 2000 and 0800 hr), daytime downstream migrants (0800–2000 hr), and upstream migrants and are shown along with the seasonal fluctuations in water level and water temperature. Arrows indicate the dates between

the first two of which overlapped the two large peaks in downstream movement. The third and largest occurred in mid-July at a time when the downstream run was almost over. Daily counts were terminated on August 5, when it appeared the major movements of fry were past. Counts were made occasionally up to August 30 but very few fry were moving.

The existence of surges in both the downstream and upstream movements of fry and their close correspondence on two occasions during 1965 is especially striking. Water levels in the Upper Babine River are rarely subject to sudden changes and have little effect on either the velocity or turbidity of the river. The changes that did occur during the period of this study had no apparent effect on the periodicities of fry movements. The Upper Babine River is, however, subject to very marked fluctuations in water temperatures and the 1965 data suggest a relation of these fluctuations to the seasonal periodicity of both downstream and upstream movements. All of the major movements occurred during periods of relatively high water temperatures.

Figure 6 illustrates the seasonal periodicity of downstream movement in fry having external yolk sacs. The percentage of yolk-sac fry in the total daily trap catch varied considerably throughout both seasons. In interpreting

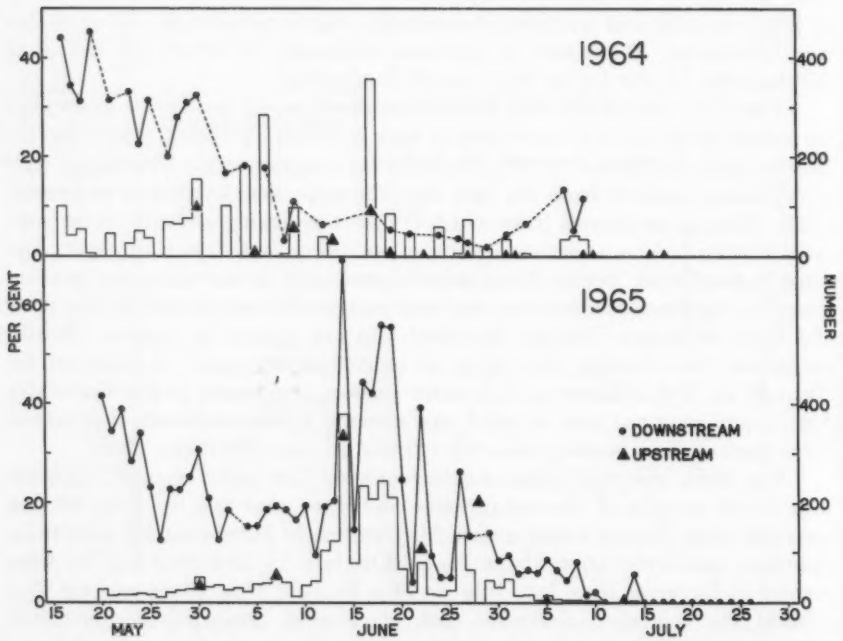

FIG. 6. Seasonal periodicities of movements of yolk-sac fry in the Upper Babine River during 1964 and 1965. Downstream data are presented as the numbers of yolk-sac fry captured daily (columns) and as the percentages of yolk-sac fry in the total daily catches (curves). The triangles give the percentages of yolk-sac fry in various samples of upstream migrants.

seasonal changes in the percentage of yolk-sac fry in the catches, two factors must be considered: first, the relative availability of yolk-sac and fully developed fry and second, the possibility of differing responses to environmental variables in fry of the two types. The high incidence of sac fry at mid-May of both years probably reflects the fact that at this time of year there are few completely developed fry in the gravel and not that there is a greater tendency for yolk-sac fry to emerge early in the season at low temperatures. The slight increase in the proportion of yolk-sac fry at the end of the 1964 season and the large peak in mid-June 1965, were both associated with temperature surges. The 1965 peak occurred several days later than the peak movement of fully developed fry during this surge so that yolk-sac fry were most abundant when the numbers of fully developed fry were declining. Yolk-sac fry continued to move downstream in considerable numbers for several days after water temperatures had fallen. The difference of several days in the timing of the peaks of yolk-sac and other fry may represent the time it takes for the former to reach the gravel surface. Yolk-sac fry were still present in the 1964 trap catches when trapping ceased but in 1965 none were taken after July 15 though trapping continued for several additional weeks.

Changes in the proportion of yolk-sac fry in samples of upstream migrants tended to parallel those in the downstream catch, though at a lower level. Considerable numbers of fry with yolk sacs moved upstream in 1965 during the peaks of abundance in the downstream samples. The difference in the degree of development of upstream and downstream migrants is actually greater than indicated because only the presence of an externally visible yolk sac is shown and not the degree of absorption, which was considerably greater in upstream migrants. Over the 2 years of the study, nearly 20% of the downstream migrants sampled had yolk sacs classified as "large" whereas only 5% of the upstream migrants fell into this category, most of these early in the season.

In 1965, a few fry with small yolk sacs were captured about a quarter mile up the North Arm on June 7 and June 15, 1.9% and 0.7% respectively of the total samples. None were taken at this location later in the year and none were ever taken further uplake.

Much remains to be done in identifying the factors affecting the seasonal periodicity of emergence and downstream movements in sockeye fry. There is a broad relationship between the seasonal periodicity of emergence and the spawning pattern of the parent population. At Karluk Lake, Alaska, for instance, the bimodal spawning periodicity of summer and fall spawners in Meadow and Canyon Creeks is mirrored in early and late peaks of downstream fry movement; in Grassy Point Creek where there is only a single summer spawning peak, there is also a single seasonal peak of fry outmigration (C. J. DiCostanzo, personal communication). Various studies of downstream fry migrations in streams at Lakelse and Babine Lakes suggest a direct relationship between the length of the parental spawning period and the seasonal duration of downstream fry movements. However, very little is known about

the factors which result in the short term fluctuations in emergence and downstream movement. Three possibilities are light intensity, stream discharge, and water temperature.

Northcote (1962) observed that larger numbers of rainbow trout fry tended to move downstream on foggy or overcast nights than on bright moonlight nights. In trough experiments with sockeye fry, McDonald (1960) got exactly the opposite result. However, many of McDonald's fry had been holding over the gravel during the day before the experiment and their behaviour may not have been that of fry emerging directly from the stream bottom. The effect of natural variations in nocturnal illumination has not, to my knowledge, been otherwise investigated.

In some streams, where changes in river discharge and turbidity are quite marked, the periodicity of fry movement is most closely related to total river discharge. At Babine Lake, the Fulton River (Fig. 7) and Gullwing Creek

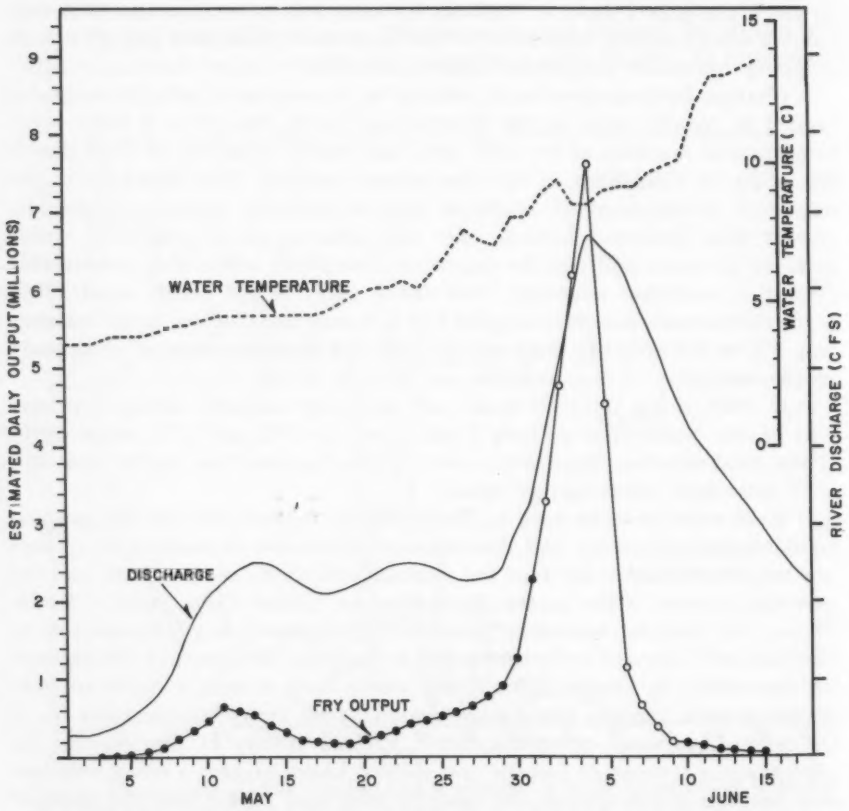

FIG. 7. The seasonal periodicity of downstream fry movement in the Fulton River, 1964.

(Withler, 1952) are of this type. Some of these fry are undoubtedly removed from the gravel by scouring but there is some evidence that the mere presence of mud and silt in the water percolating through the gravel will stimulate increased activity and emergence in sockeye fry even when water levels and temperatures are relatively constant (Coburn and McCart, 1967).

Most studies of downstream movement in sockeye fry have been done in small tributary streams in which temperature fluctuations are much smaller than those in the Upper Babine River and in which fluctuations in other environmental variables complicate the pattern of emergence. There is consequently no clear evidence of thermal regulation of emergence and downstream movement comparable to that for the relatively stable Babine River.

The available evidence suggest that, once the fry have reached a certain stage of development, anything that stimulates them to increased activity—changes in water quality, sharply increased temperatures, dark nights—will bring them out of the stream bottom in large numbers.

The increase in upstream movement with rising water temperatures also appears to be the result of activity stimulation. Runnström (1957) and Northcote (1962) found similar relationships in brown trout and rainbow trout respectively. Both authors were impressed by the fact that, after a sharp increase in upstream movement coincident with a rise in water temperature, fry counts often fell even though water temperatures continued to rise. Runnström suggests that this may occur when temperatures exceed the level for maximum activity (10–12 C in brown trout, Brown, 1946). Northcote (1962) felt that in many instances such declines were the result of a decreased "supply" of animals ready for upstream movement. Brett et al. (1958) found that the performance of sockeye fry in swimming tests reached a maximum at 15 C. This temperature was rarely exceeded during the temperature peaks in the Upper River and it would therefore appear that the early declines in the number of fry moving upstream were primarily the result of a depleted supply.

DIEL PERIODICITY OF FRY MOVEMENTS

Figure 8 illustrates the hourly distribution of nocturnal downstream trap catches and upstream grid counts for six representative periods in 1964 along with data on light intensity and water temperatures.

The periodicity of nocturnal downstream migration assumed the sharply peaked form previously described by McDonald (1960) and Hartman et al. (1962) in other populations of sockeye fry. Peak captures were made in the hours immediately after dark, and numbers declined thereafter to very low levels shortly after sunrise. Light intensity appeared to be of primary importance in regulating this periodicity. McDonald (1960) and Hartman et al. (1962) (op. cit.) found the relationship of downstream movement and light intensity to be so close that the nightly period of downstream movement became progressively restricted as the season advanced due to changes in

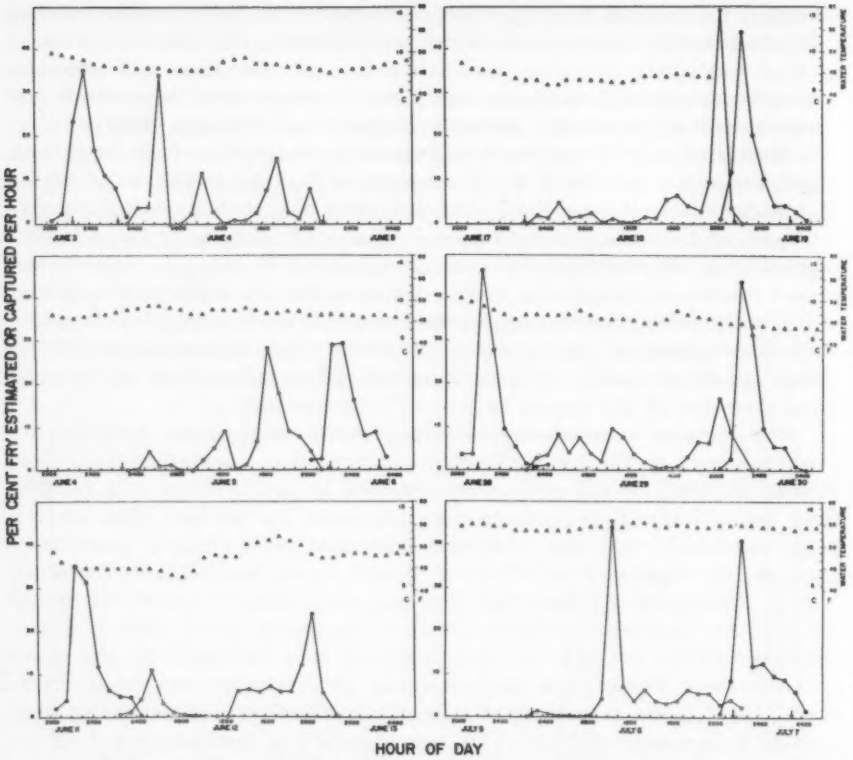

FIG. 8. The diel periodicity of fry movements in the Upper Babine River during six representative periods in 1964. The data are shown as percentages of the total downstream (black circles) and upstream (white circles) movement occurring in each hourly period. The mean hourly temperature is shown by the triangles. The arrows indicate, where data is available, the times at which light intensity reached .01 ft-c in the evening and rose above it in the morning.

the hours of darkness. At the same time, the peak of nocturnal migration came progressively later.

To determine whether similar seasonal trends were characteristic of the diel periodicity of downstream movement in the Upper Babine River and to partially obviate variability due to chance fluctuations in environmental factors, the data have been grouped by 1-week periods and are presented in Fig. 9 as the average percentages of the largest single hourly catch that were taken between 2000 and 0500 hr (2000–0400 hr for the week May 31–June 6).

There are two definite trends: first, a tendency for the distribution of catches to become more sharply peaked; second, a shift in this peak from the period 2300–2400 hr to an earlier period, 2200–2300 hr. These trends continue through the summer solistice (June 21). The latter trend is the reverse

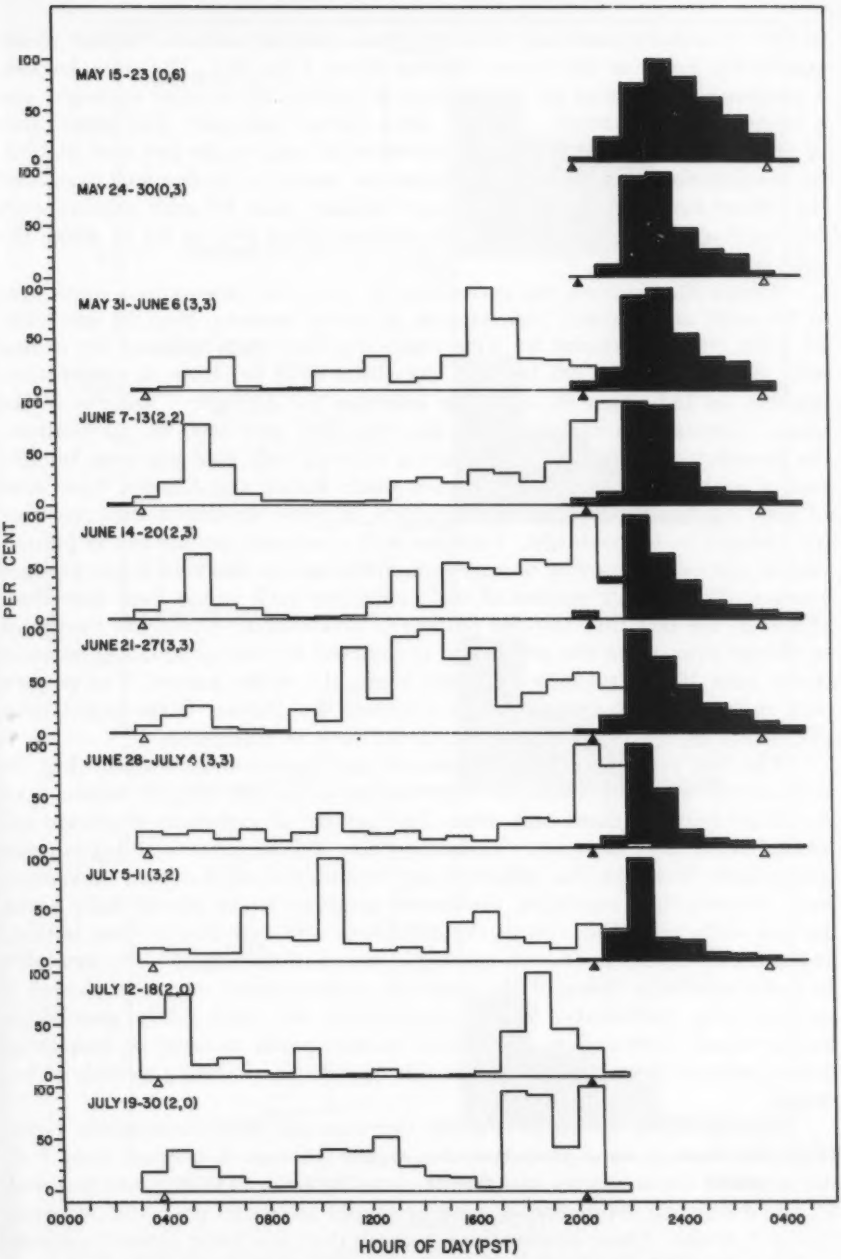

FIG. 9. Weekly changes in the diel periodicity of fry movements in the Upper Babine River during 1964. The data for each hour are expressed separately for downstream (black columns) and upstream (white columns) movements, as the average percentage of the largest single hourly catch. The numbers in parentheses indicate first, the number of days in each week for which upstream data are available and second, the number of nights for which downstream data are available. The triangles indicate the average times of sunrise and sunset during each week.

of that previously described for downstream migrant sockeye. Similar trends apparently occur in the Lower Babine River (Fig. 10). The data indicate a progressive decline in the importance of catches taken after midnight and a corresponding increase in those taken before midnight. The significance of this pattern is not clear. It may be related in part to the fact that in 1964, fry moving downstream early in the season tended to be less well developed than those moving later. There is some evidence that fry with external yolk sacs have a diel pattern of emergence different from that of fry in which the yolk is fully enclosed.

Figure 11 compares the periodicity of nocturnal downstream movement of fry with and without yolk sacs on 4 nights between May 16 and May 29, 1964. Fully developed fry were more abundant than yolk-sac fry during both the first (2100–2200 hr) and last (0300–0400 hr) hour of observation, possibly an indication of a greater tolerance for daylight. Captures of the former increased to a sharp peak between 2300 and 2400 hr. In contrast, the periodicity of captures of fry having external yolk sacs was more broadly peaked with nearly equivalent catches made during the 3 hours from 2200 to 0100 hr. Finally, a greater proportion of fully developed than yolk-sac fry emerged before midnight. Together with a seasonal decline in the proportion of yolk-sac fry moving downstream, differences of this kind might produce a seasonally changing pattern of diel periodicity such as has been described. However, the fact that the diel pattern of downstream movement continued to change even when the proportion of yolk-sac fry remained fairly constant (from June 10 on) suggests that this is not the whole answer. The pattern may reflect some major difference between the Babine River populations and others in the behaviour of fry at the time of emergence.

The diel periodicities of downstream and upstream movement (Fig. 8) were nearly mutually exclusive with the latter almost entirely confined to the hours between dawn and dusk. The pattern of upstream migration exhibited much more day-to-day variability than did the pattern of downstream movements. Although the initiation and termination of upstream movement were certainly light regulated, the factors resulting in the several daily peaks are not easily identified. One of the difficulties with this kind of data is that, as Northcote (1962) points out, nothing is known of the supply of fry available to move upstream. None of the observed environmental variables showed a corresponding periodicity. Water temperature, the most likely possibility, was obviously unrelated to hourly grid counts: peaks occurred as frequently during periods of stable or decreasing temperatures as during periods of increase.

When grouped by weeks (Fig. 9), the data still show considerable variability but there is some indication of a regular pattern. During at least 5 of the 8 weeks there was an identifiable morning peak of movement followed by a mid-morning depression; a more prolonged afternoon peak was apparent during 6 weeks. These observations suggest that the basic activity pattern of upstream migrants was bimodal. Hoar (1958) described a midday depres-

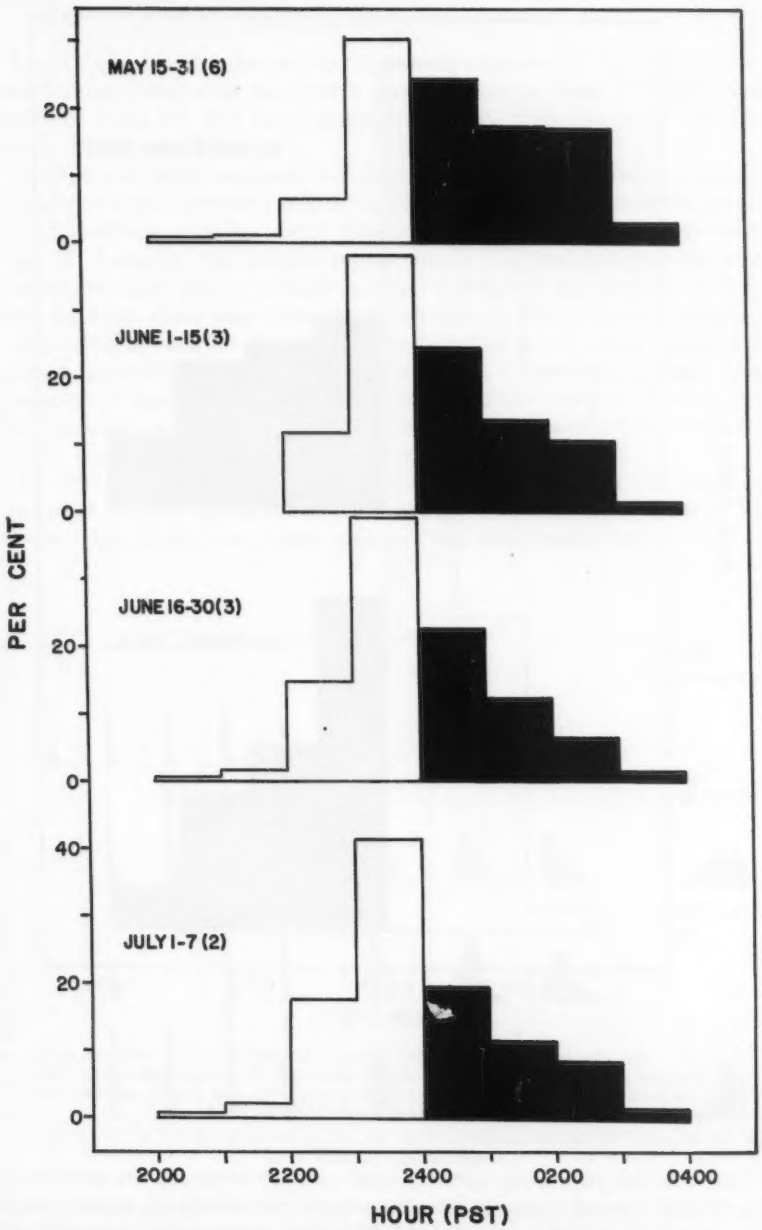

FIG. 10. Biweekly changes in the diel periodicity of downstream movement in the Lower Babine River during 1962. The data are presented as the percentage of the total nightly catch moving downstream during each hourly period from 2000 to 0400 hr (2200 to 0400 hr from June 1–15). The hours before and after midnight are distinguished to facilitate comparison. The number in brackets indicates the number of nights during each 2-week period for which data are available.

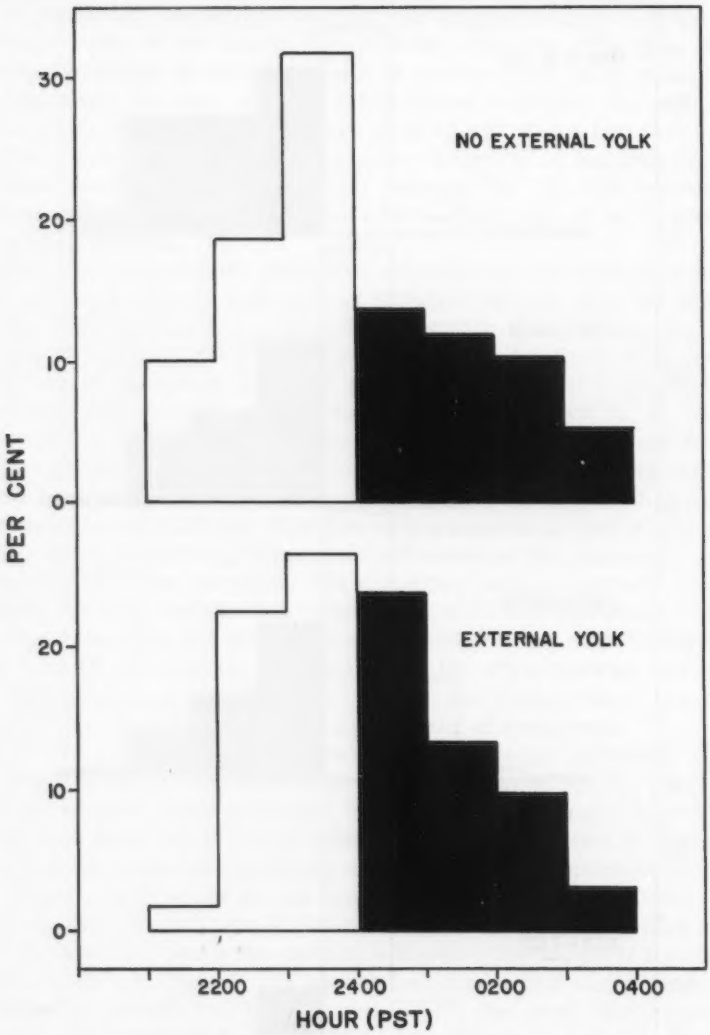

FIG. 11. Periodicities of nocturnal downstream movement of fry with and without externally visible yolk sacs. The data, expressed as percentages of the total captures between 2100 and 0400 hr, are the averages for the hourly catches on 4 nights between May 16 and May 29, 1964.

sion in both the swimming activity and current responses of sockeye fry. Groot (1965), commenting on the dawn–dusk periodicity of activity in migrating smolts notes "There is some evidence that this is the basic diel activity rhythm of sockeye salmon, young or old, migrating or not."

LENGTH-FREQUENCIES

Length-frequency information is most complete for fry taken in the Upper Babine River and the North Arm of Babine Lake. The length-frequencies of these fry will be considered first and then compared with those of fry in the Lower River.

In 1964, the major emphasis was on a comparison of the length-frequencies of downstream and upstream migrants. The length-frequencies of the samples, taken at intervals of a few days, grouped by weekly periods, are presented in Fig. 12. Initially, the length–frequencies of the two groups were similar but although there was a marked seasonal increase in the lengths of the upstream samples, there was virtually no change in the length composition of the downstream samples other than the occurrence of a few larger fish in samples taken during the last week of the season. A comparison of the length-frequencies of downstream migrants and fry taken from the stream gravel during the same period (May 22 to May 26, 1964) revealed that the downstream migrants were larger and better developed (Fig. 13)

In 1965 also, there was a seasonally increasing disparity in the lengths of downstream and upstream migrants (Fig. 14). Once again, a few larger fry were taken during the latter stages of the downstream run.

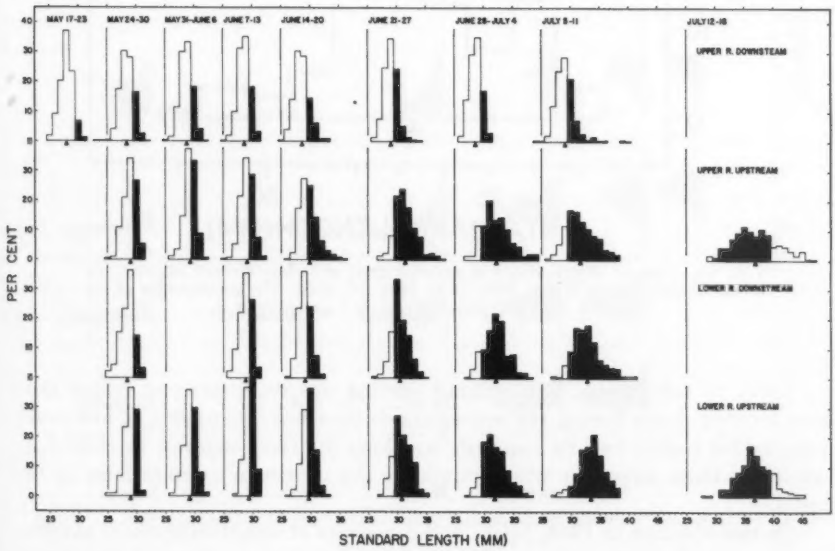

FIG. 12. Length distributions of fry captured in the Upper and Lower Babine rivers during 1964 expressed as the percentage of the total sample in each millimeter length class. The percentages between 30 and 40 mm have been blackened to facilitate visual comparison of samples taken on various dates. The triangles indicate the mean lengths of samples.

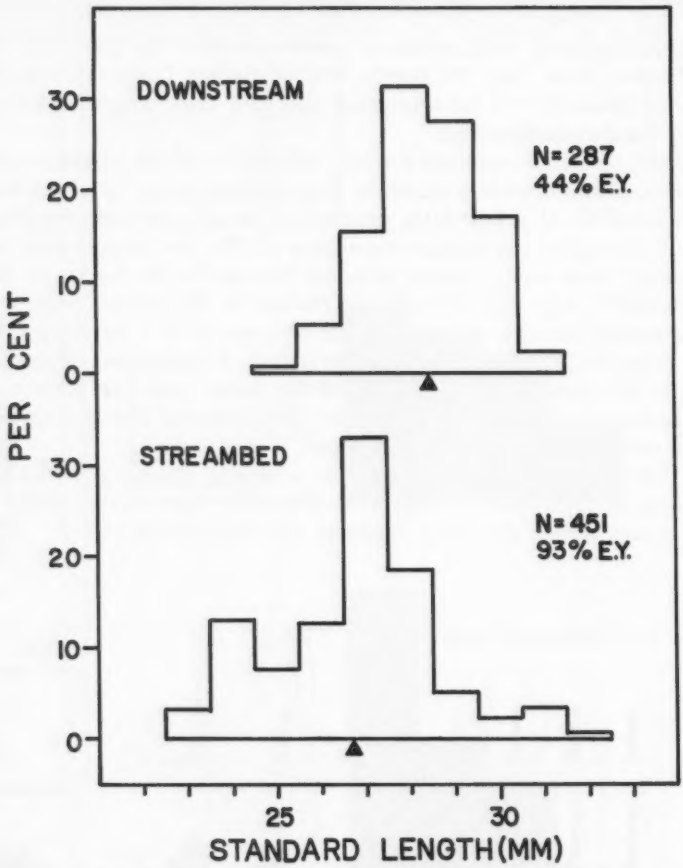

FIG. 13. Length distributions of pre-emergent and downstream migrant fry in the Upper Babine River, May 22 to May 26, 1964. The percentages of fry having external yolk sacs (E.Y.) are indicated.

Early in the season, fry captured moving upstream were no larger than those moving down during the same period. However, many fry, if not most, remained for a time feeding and growing along the river edges or in Nilkitkwa Lake. It is these larger fry which dominate the upstream samples later in the season.

On one occasion in 1964, July 2 and 3, a series of length-frequency samples was taken in the Upper Babine River and along the east shore of the North Arm of Babine Lake (Fig. 15). There was a direct relationship between the mean length of fry and distance uplake, exactly what would be expectde were fry dispersing from a single source and growing as they dispersed. In

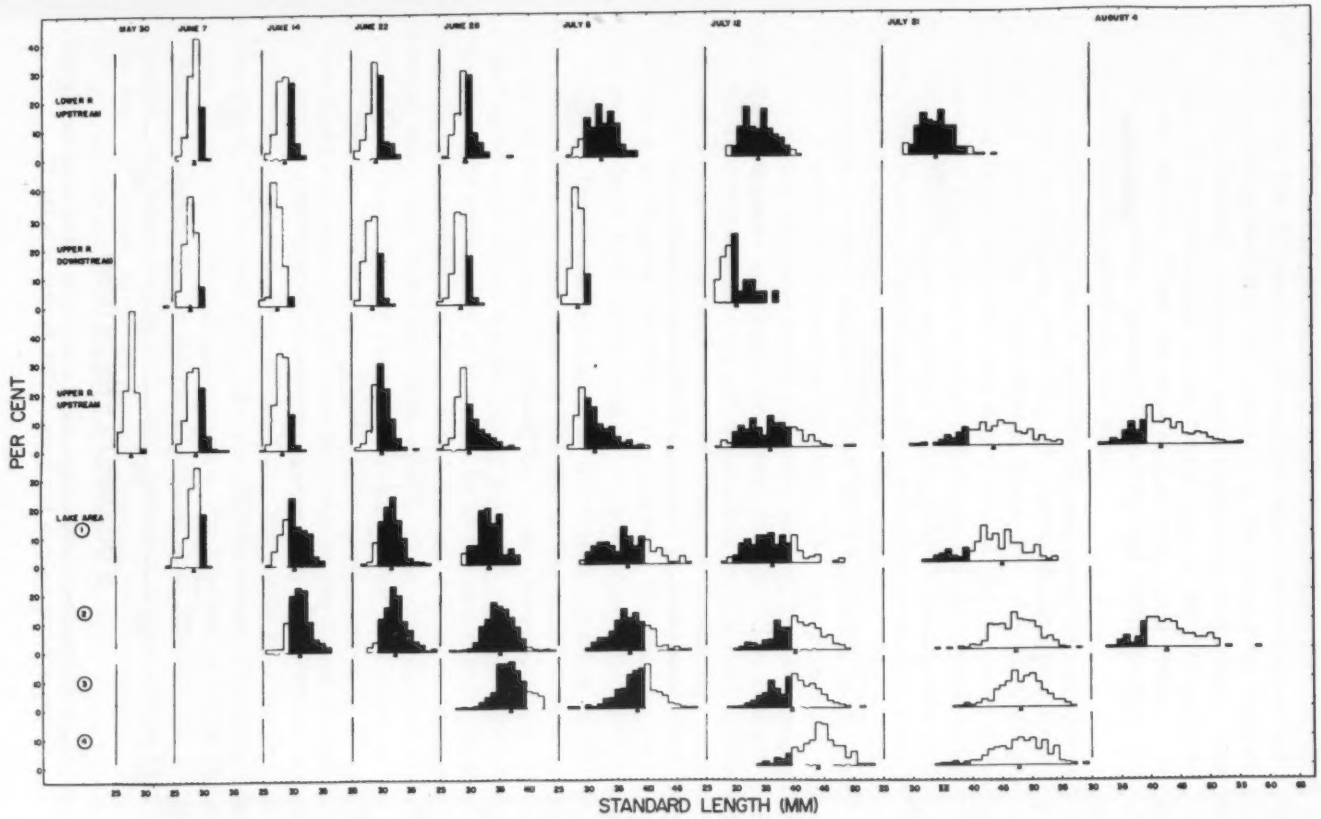

FIG. 14. Length distributions of sockeye fry captured in the Upper and Lower Babine rivers and the North Arm of Babine Lake during 1965. Format as in Fig. 12.

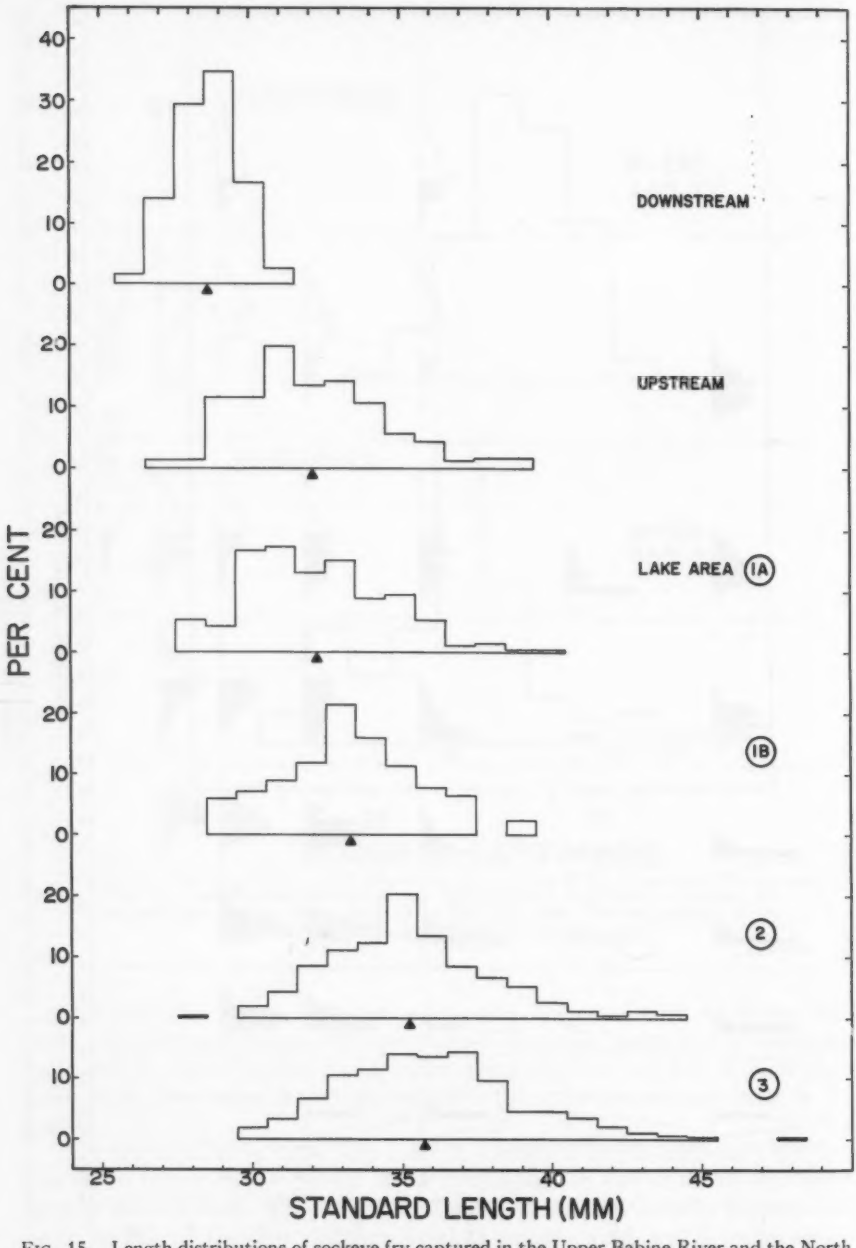

FIG. 15. Length distributions of sockeye fry captured in the Upper Babine River and the North Arm of Babine Lake on July 2 and 3, 1964. Format as in Fig. 12.

1965, North Arm samples showed a similar relationship up until early July (Fig. 14). On July 12, however, the pattern began to break down and on July 21 the length-frequencies of fry collected in the three areas farthest uplake were almost identical, suggesting that considerable mixing had taken place. By August 4, fry were scarce in the littoral area of the lake and were taken in only one locality. These fry were smaller than those taken in the same area the previous week. Apparently most fry had abandoned the lake edges and become entirely pelagic by this time.

The length-frequencies of downstream and upstream migrants captured in the Lower River during 1964 (Fig. 12) were more alike than those of comparable fry taken in the Upper Babine River. Fry of both kinds gradually increased in length and there was only a hint of the increasing disparity so obvious in the Upper River samples. There are several possible explanations for this. In the first place, spawning in the Lower River precedes that in the Upper River by several weeks and there is some suggestion that this difference is reflected in the respective periods of fry emergence. This would account for the almost complete absence of small fry in collections made during the last 2 or 3 weeks of downstream sampling. The high proportion of larger fry taken during this same period may have resulted from the comparatively difficult environment with which the Lower River fry must contend. In this river, where currents are strong and where there are few good holding areas, even well-developed fry might have difficulty in maintaining position. A particularly unfavourable location is an island in midstream about 100 m above the weir. Many of the large fry captured at the fence may have been those swept down while attempting to move upstream from this island.

In 1964, the length-frequencies of fry moving upstream in the Upper and Lower Babine rivers were similar during the period from May 24 to July 18. In 1965, the mean lengths of fry in the two groups (Fig. 14) were similar up through July 5 but the Upper River fish were considerably larger thereafter. The explanation of this may lie in the fact that fry moving upstream in the Lower Babine River spend the period between emergence and eventual entry into Nilkitkwa Lake entirely along the edges of the river. On the other hand, several lines of evidence suggest that many of the fry moving upstream in the Upper Babine River spend a period of time in Nilkitkwa Lake and that some of them originate in the Lower Babine River. For instance, large upstream migrations were observed on several occasions in 1965 the day after surveys indicated that there were very few fry holding along the edge of the river itself. This was after the period of significant downstream catches and the sudden increase in the numbers of fry in the river could not have been due to the recruitment of newly emerged fry. On other occasions, continuous bands of fry have been seen moving out of Nilkitkwa Lake into the Upper River (F. Jordan, personal communication). The fry moving upstream on these occasions were often very large. Further evidence of a movement out of Nilkitkwa Lake comes from townetting studies which

indicate that sockeye populations in Nilkitkwa Lake decline precipitously from June to October (F. Jordan, personal communication).

In both the Upper and Lower rivers, fry taken during 1964 averaged slightly larger than those taken during comparable time periods in 1965 (Fig. 16).

LENGTH–WEIGHT RELATIONSHIP

The mean weights of fry in each millimeter length class were used in calculating the length–weight relationships of sockeye fry in the Upper and Lower Babine rivers and the North Arm. The means and the calculated regression lines are illustrated in Fig. 16. Although the mean weights of each length class are indicated, only data for fry 31 mm or longer were used in calculating the regression statistics because smaller fry appeared, in some instances, to have a markedly different length–weight relationship. This difference is thought to be due to the presence of yolk in many of the smaller fish, particularly those most recently emerged.

Figure 17 indicates the percentages of fry in a total sample of 375 fry between 25 and 35 mm in which the yolk was externally visible in the form of a yolk sac and in which vestiges of the yolk could be identified internally after the body cavity had been opened. Many fry in which the yolk sac had been completely enclosed retained some yolk internally. All of the 25-mm fry examined contained yolk material. In 46% of these, the yolk sac was visible externally. Between 25 and 30 mm there was a sharp decline in the propor-

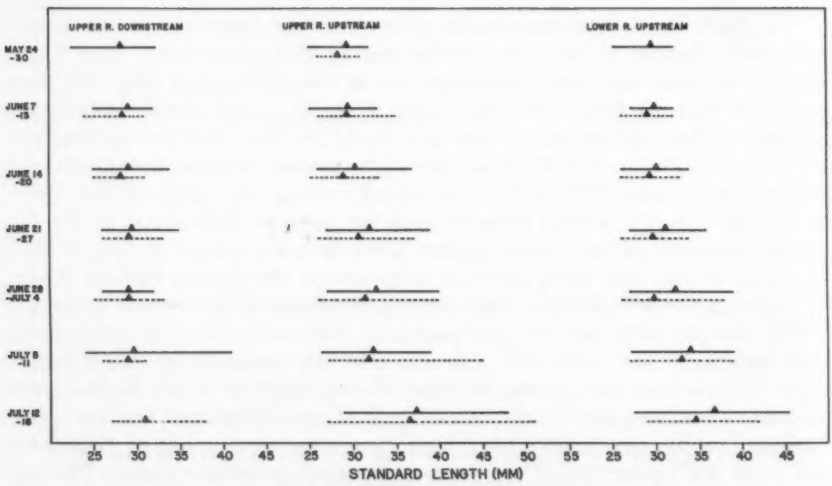
UPPER R. DOWNSTREAM
UPPER R. UPSTREAM
LOWER R. UPSTREAM
MAY 24 -30
JUNE 7 -13
JUNE 14 -20
JUNE 21 -27
JUNE 28 -JULY 4
JULY 5 -11
JULY 12 -18
25 30 35 40 45 25 30 35 40 45 50 55 25 30 35 40 45
STANDARD LENGTH (MM)

FIG. 16. Length ranges and mean lengths (triangles) of sockeye salmon fry captured in the Upper and Lower Babine rivers during comparable periods in 1964 (solid lines) and 1965 (broken lines).

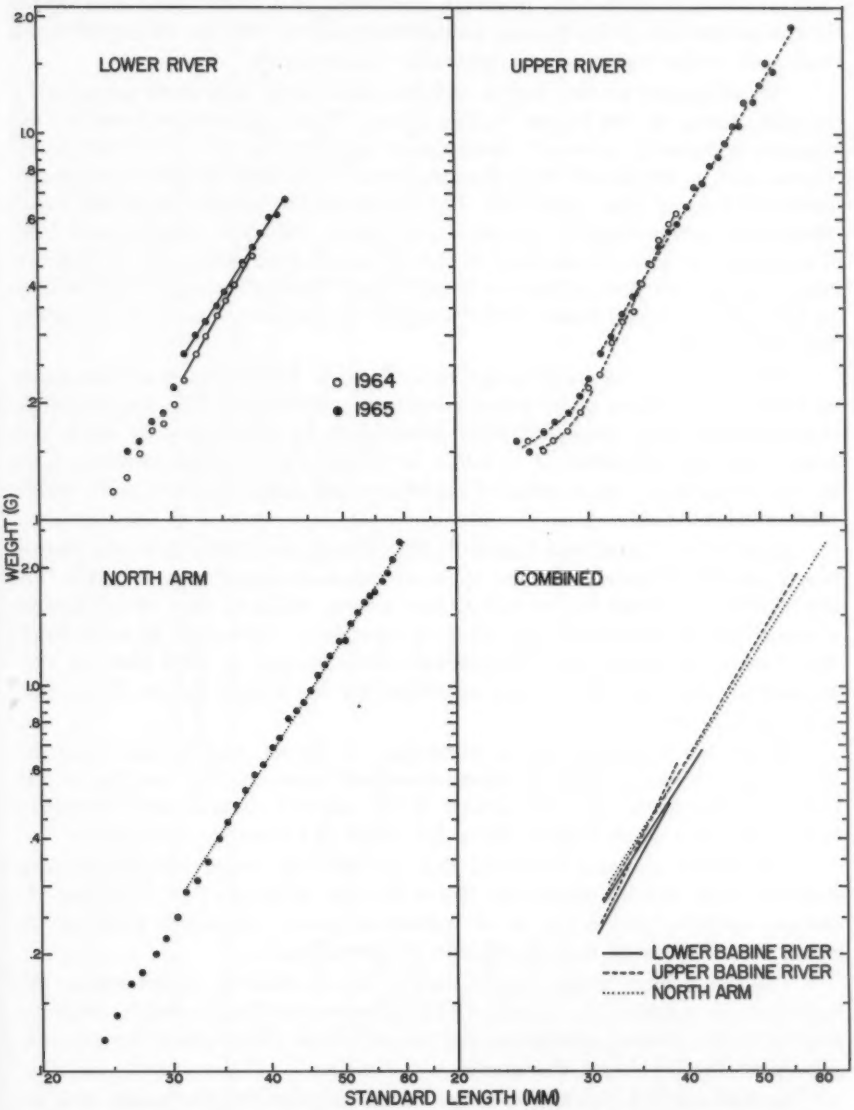

FIG. 17. Length–weight relationships of fry captured in the Upper and Lower Babine rivers during 1964 and 1965 and in the North Arm of Babine Lake during 1965. The regression lines are reproduced together in the lower right quadrant.

tion of fry containing yolk material, paralleled at a lower level by a decline in the proportion of fry having an external yolk sac. No fry 31 mm or larger had yolk either externally or internally discoverable.

An inflection in the length–weight relationship was most apparent in samples taken in the Upper Babine River. These samples included a large number of recently emerged, downstream migrants in the sub-31 mm length classes and, as the dotted trend lines indicate, fry of these lengths were proportionately heavier than larger fish. The length–weight samples from the Lower River were predominantly upstream migrants in 1964 and entirely so in 1965. The length–weight relationship of sub-31 mm Lower River fry is closer to that of larger fish than is the case in the Upper River. The lengths and weights of the well-developed North Arm fry appear to fall on the same line on either side of 31 mm.

The regression formulae are given in Table I. Also included are the results of some comparisons made using covariance techniques. The length–weight relationships being compared were tested first for differences in slope and, where these did not differ ($P = 0.01$), for differences in adjusted mean. Each of the comparisons made revealed significant differences in the length–weight relationships tested.

In both the Upper and Lower Babine Rivers, the 1964 fry in the shorter length classes weighed less than those in the same size-classes in 1965. The distribution of points in the sub-31 mm groups suggests that this difference was already established at the time of emergence. However, in both rivers the increase of weight with length was much greater in 1964 than in 1965 so that the lines for the 2 years converge; for the Upper Babine River they eventually cross.

When the length–weight relationships of Upper and Lower River fry are compared within years a common pattern emerges. The weights of fish from the two rivers are very similar at 31 mm but diverge with increasing length. In both years Upper River fry weighed proportionately more.

Covariance analysis indicated that in 1965 the length–weight relationships of Upper Babine River and North Arm fry differed ($P = 0.01$) but the obvious irregularities in the fit of individual points, especially those of the longer length classes, suggest caution in interpretation.

The significance of the length–weight data is difficult to determine. We have first to consider the factors which influence the length–weight relationship up to the time of emergence and second, those which affect the relationship after the fry leave the gravel.

As indicated, in both rivers, fry of the shorter length classes weighed less in 1964 than fry of the same lengths in 1965. There is also some evidence (Fig. 16) that in both rivers the mean lengths of fry were slightly greater in 1964 than in 1965. Possibly the factors which produced the observed differences in the lengths and weights of the two year-classes of fry were common to both rivers.

TABLE I. Results of linear regression and covariance analysis of logged length–weight data for fry captured in the Babine River and the North Arm of Babine Lake.

Locality	Year	Intercept (a)	Slope (b)	L.R.[a] '64		L.R. '65		U.R. '64		U.R. '65		N.A. '65	
				S.	A.M.[b]	S.	A.M.	S.	A.M.	S.	A.M.	S.	A.M.
Lower River	1964	−5.0059	3.6092	–[c]	–	**	–	*	**	–	–	–	–
	1965	−4.1238	3.0551	**[d]	–	–	–	–	–	**	–	–	–
Upper River	1964	−5.8407	4.1798	*[e]	**	–	–	–	–	**	–	–	–
	1965	−4.5114	3.3212	–	–	**	–	**	–	–	–	**	–
North Arm	1965	−4.1843	3.1262	–	–	–	–	–	–	**	–	–	–

[a]L.R. = Lower Babine River; U.R. = Upper Babine River; N.A. = North Arm.
[b]S. = slope; A.M. = adjusted mean.
[c]– = No test made.
[d]** = Significant at $P = 0.01$.
[e]* = Significant at $P = 0.05$.

Various aspects of the physical environment have been shown to affect the growth and development of salmonid embryos: oxygen concentration, water velocity, water temperature, and daylight have been implicated in various studies (Garside, 1959; Silver et al., 1963; Shumway et al., 1964; Brannon, 1965). There is no information available about physical conditions in the Upper or Lower rivers during the winters which produced the 1964 and 1965 fry. However, one other interesting possibility has been explored: that year-to-year differences in length–weight relationship may be related to the size of eggs from which the fry develop.

Martin (1949) identified five relative growth stanzas in the life history of Atlantic salmon, delimited by inflections occuring at approximately the eyed egg stage, hatching, ossification, and sexual maturity. The length–weight relationship is a form of relative growth constant. Martin (1949) comments, "Differences in fish size, at growth inflection, is shown to be one of the factors important in the control of relative heaviness." It can be demonstrated that there probably were differences in the size of 1964 and 1965 Babine River fry at several critical periods in their early development.

Table II presents the proportions of 4- and 5-year-old female sockeye on the spawning grounds of the Upper and Lower Babine rivers during the years which produced the 1964 and 1965 fry. Females 4 years old predominated in the 1963 spawning run, 5-year-olds in 1964. Females of 4 years were considerably smaller than those of 5. Recent investigations on the Lower River have shown that there is a direct relationship of parent size to egg size and, because 4-year-old females are smaller, their eggs are, on the average, also smaller than those of 5-year-olds — .1448 and .1718 g respectively, in 1965 (T. Bilton, unpublished data). Thus, when 4-year-old females predominate, the average size of the eggs deposited in the gravel should be smaller than when 5-year-olds predominate. There is some additional information suggesting that fry resulting from large eggs may weigh proportionately more than those from small eggs

TABLE II. Age composition, and mean hypural length (H.L.) of each age-group,[a] of female sockeye salmon spawning in the Upper and Lower Babine rivers during 1963 and 1964.

		Age 4_2		Age 5_2		Age 5_3	
Location	Year	%	Mean H.L. (*cm*)	%	Mean H.L. (*cm*)	%	Mean H.L. (*cm*)
Upper River	1963	73.9	44.9	26.1	50.6	–	–
	1964	9.4	43.7	90.6	49.6	–	–
Lower River	1963	66.0	44.6	33.0	50.7	1.0	43.2
	1964	18.8	42.9	81.2	49.3	–	–

[a]The subscript indicates the number of years spent at sea.

when raised under similar experimental conditions (Fig. 18). Here then, perhaps, is a mechanism which might produce the observed year-to-year differences in the length–weight relationships of fry in the rivers. The argument does not, however, take into account that at emergence the 1965, large-egg fry were apparently shorter as well as proportionately heavier than the 1964 fry. In unpublished experiments conducted by the author and T. Bilton, fry from large eggs were consistently longer than those from small eggs when raised under similar conditions. The factors determining the length–weight relationships of emerging fry are obviously complex and egg size is certainly not only, or even the most important, consideration. It is, however, one which warrants further investigation. It might for example, explain Skud's (1955) observation that pink salmon fry migrating out of Sashin Creek during March weighed proportionately more than those migrating later, in April and May. He comments, "Data gathered from Sashin Creek show that in six out of seven years the early

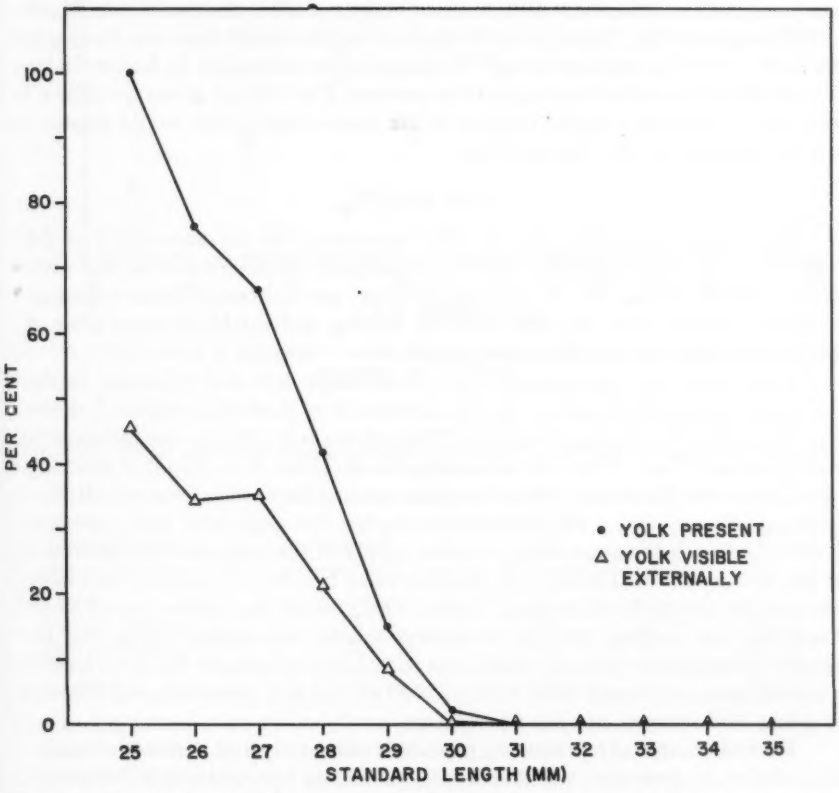

FIG. 18. Relationship of yolk absorption to fish length in a sample of 375 fry from the Upper Babine River, 1965.

spawners were larger than late spawners. The resulting progeny may reflect this adult condition." Possibly the *female size/egg weight* and *egg weight/fry weight* relationship are similar to those indicated for Babine River sockeye salmon.

The factors which govern the increase of weight with length after emergence are also little understood. Parker and Vanstone (1966) give some indication of the complexity of this problem as it applies to pink salmon fry. In the Babine rivers there were differences between year-classes in the same river and differences in the same year-class in different rivers. There is no obvious explanation for the relatively high rate of weight gain in the 1964 fry of both the Upper and Lower Babine rivers. Water temperatures were slightly lower in 1964 (9.4 C — May 25 to July 17, average) than in 1965 (9.8 C). There is no comparative data on plankton abundance during the 2 years but significant annual differences can occur in Babine Lake (Johnson, 1961). The greater rate of weight gain of Upper River fry in comparison with Lower River fry of the same year-class may reflect differences in the energy budget of fry in the two rivers. Martin (1949) suggests that, "changes in the slope of length–weight lines may be effected by such factors as malnutrition." Plankton is less abundant in Lower Babine River and water velocities consistently greater. The balance of energy inflow in the form of food and energy outflow in the form of swimming would appear to favour those fry in the Upper River.

FOOD HABITS

On June 3/4 and June 17/18, 1965, samples of 10 fry were taken at 2-hr intervals over a total period of 24 hr in an attempt to determine the diel periodicity of feeding (Fig. 19). It was assumed that the numbers of intact organisms would be highest when fry were actively feeding and would decrease when digestion exceeded the intake of new material.

Results for the two periods vary considerably but they do agree in that the major peaks occur during the day and the lows during the night. A dotted line illustrates the average tendency. The pattern is similar to one obtained by Northcote and Lorz (1966) for kokanee in Nicola Lake, B.C., and that described by Parker and Vanstone (1966) for pink salmon in Burke Channel, B.C.: a single afternoon peak with a depression during the night and early morning. It contrasts with the apparently bimodal nature of the upstream fry movement in the Babine River. Possibly the periods when fry are not actively swimming are devoted primarily to feeding. Groot (1965) found that under experimental conditions the feeding activity of sockeye smolts was highest when the frequency of behaviour patterns associated with active migration was low; feeding occurred most commonly from 1200 to 2000 hr and was preceded and followed by peaks in fluttering and position change.

The yolk material represents a portable food supply and it was of considerable interest to determine whether fry began feeding before this was completely absorbed. Table III records the results of the examination of two selected samples: 52 fry taken at 1300 hr, June 17, and 48 fry taken at 0100 hr, June 18,

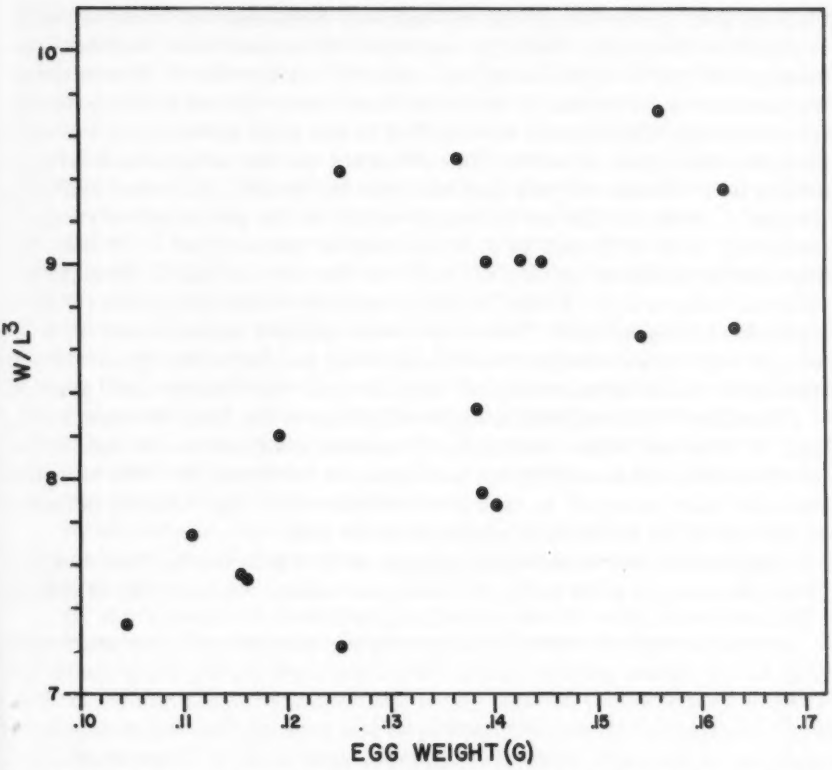

FIG. 19. Relationship of egg weight to the condition factor (W/L^3) of the resultant fry. Data courtesy of T. Bilton.

TABLE III. Percentages of stomachs containing food, in fry with and without remaining yolk material.

Date and time	No. examined	With food (%)	Average length (*mm*)
With yolk			
June 17, 1300 hr	26	34	27.5
June 18, 0100 hr	24	45	27.3
Total	50	40	27.4
Without yolk			
June 17, 1300 hr	26	96	30.5
June 18, 0100 hr	24	95	28.2
Total	50	96	29.4

1965. In both instances half the fry examined contained yolk material, either externally or internally. Both day and night, the proportion of stomachs containing food was far higher among non-yolk than among yolk fry. Thus, although the presence of yolk material within the body cavity did not preclude feeding, fry containing yolk material did not feed to the same extent as fry in which the yolk was entirely absorbed. This difference was not simply the result of a difference in the size of yolk and non-yolk fry. Small (<28 mm) and large (28 mm +) yolk fry did not differ appreciably in the percentage of stomachs containing food: 36% and 44% respectively of two samples of 25 fish. The mean size of the latter group (28.2 mm) was the same as that of the nighttime sample of non-yolk fry (Table III) which had a very high proportion (95%) of stomachs containing food. There are several possible explanations. Yolk fry may be very recent emergents which have not yet had much opportunity to take food, or the presence of yolk may in some way inhibit their appetite.

Foerster (1925) reported marked differences in the food of sockeye yearlings in lakes and those in streams; crustacean plankters in the former and insects, chiefly chironomid larvae and pupae, in the latter. In 1965, numerous stomachs were examined to determine whether there was a similar difference in the food of fry in the river and those in the lake.

Information on the stomach contents of fry taken during June and July 1965 in five areas is given in Fig. 20. Estimated volume was used only to indicate

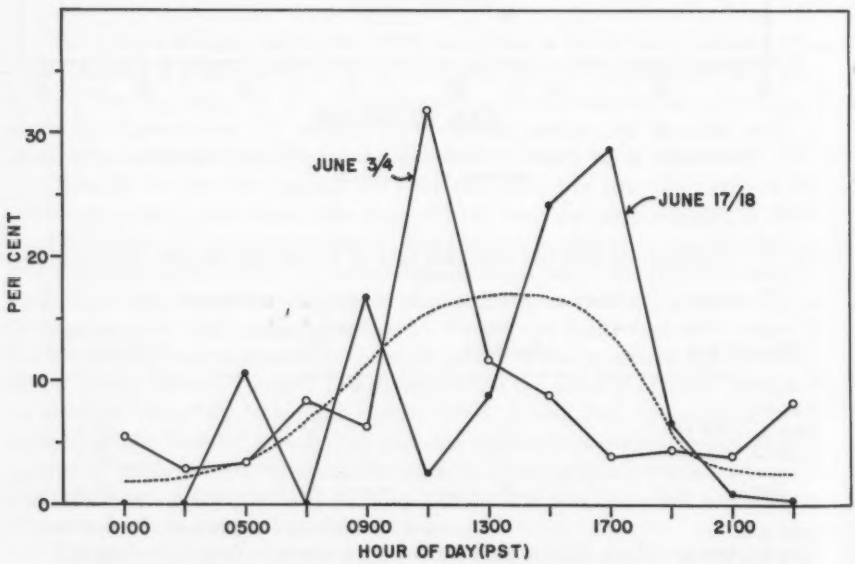

FIG. 20. Percentage of the total identifiable food organisms occurring in each of 12 samples taken at 2-hr intervals from 1300 hr June 3 to 1100 hr June 4 and from 0900 hr June 17 to 0700 hr June 18. The data have been plotted from midnight to midnight, not in the order in which samples were taken. The dotted line indicates the general trend.

the importance of those insects and plankters which were too far digested to be identifiable as individuals and to compare the total volume of insects with the total volume of plankters. The data labelled "Upper River Downstream" included both day and night downstream migrants removed from the trap at the regular morning and evening counting times. These had all had some experience of daylight and were not necessarily representative of fry migrating during the nightly peak of downstream movement. These data might best be viewed as an indication of the food items which constitute the first "meals" of recently emerged fry. That these were predominantly recently emerged fry is indicated by the nearly 50% of the stomachs which were empty, the only samples in which a significant proportion of empty stomachs occurred.

Insects did not exceed 8% of the total number of food items in any samples. However, they appeared in a relatively high percentage of the stomachs. Also, because they were individually large, they constituted a relatively large percentage of the total food volume. Insects were numerically most important in the Upper Babine River. Volumetrically, the Upper and Lower river and the North Arm inshore samples were very similar. Insects were of least importance in samples taken offshore in the North Arm.

In all samples, dipterans of the family Chironomidae were the most important insect food. Among these, pupae occurred more frequently than either larvae or adults.

By every measure, crustacean plankters were the most important food of the young fry. The copepods *Diaptomus* and *Cyclops*, in that order, were the mainstay of the diet of fry in the rivers and, to a lesser extent, in the North Arm of Babine Lake. Immature and partly digested individuals of these two genera were not distinguished and have been grouped as "unassigned *Cyclops* and *Diaptomus*." A third large copepod, *Heterocope*, appeared in only small numbers in stomach samples taken in the rivers but was of considerable importance in offshore townet samples, occurring widely in relation to its numbers. The intake of the cladoceran plankters *Daphnia* and *Bosmina* in the rivers was small in comparison with that of the copepods. However, in North Arm inshore samples, *Bosmina* was taken in fair numbers and occurred in over 40% of the stomachs. Offshore, *Daphnia* was second only to *Diaptomus* in total numbers of identifiable organisms and occurred in more stomachs than any other plankter; *Bosmina*, though not abundant, also occurred widely.

Except for the North Arm offshore samples there was fair agreement between the relative abundance of individual plankters in plankton samples and their relative abundance in fry stomachs. An exception was *Heterocope*, which appeared in small numbers in stomachs taken in the rivers but was not present in any of the plankton samples from these areas.

Offshore in the North Arm, both *Heterocope*, which was not present in the plankton samples, and *Daphnia*, present in very small numbers, formed a considerable part of the stomach contents. However, sockeye fry in Babine Lake are thought to undergo a daily vertical migration from the deeper water, which they occupy during the day, toward the surface (Johnson, 1961). This

movement takes place at dusk. It was at this time that the surface townet catches were made. As a result, the plankton samples, taken from 0-5 m, may not be representative of the relative abundance of organisms at the depths at which fry were feeding. There is the additional possibility that the relative abundance of various food items is the result of selective feeding by fry. Ricker (1937) has shown that such selection does occur in sockeye fry.

The 1965 plankton samples indicate that plankton were most abundant in mid-June (Fig. 21). Throughout June, plankton abundance was considerably greater inshore in the North Arm than elsewhere. Next in order of plankton abundance were the North Arm offshore (0–5 m), Upper Babine River, and Lower River. The tendency for fry to remain inshore after entering the North Arm may be related, in part, to the abundance of plankton there.

PREDATION

From the time fry in the Upper Babine River emerge from the gravel until they leave the river and enter the lake, they are almost entirely confined to a narrow strip along the river bank. The dense populations that accumulate there appear to be very vulnerable to predation. In 1965, regular collections of predatory fishes were made throughout the period of fry migration with both beach seines and gillnets. A few were taken by angling. Collections made in the Upper River near Smokehouse Island and in the North Arm of Babine Lake (primarily in area 1) have been treated separately.

Seven species of fish were found to be preying on sockeye fry: rainbow trout, cutthroat trout, coho salmon, Dolly Varden, lake trout, lake whitefish, and burbot (Table IV). An additional species, the squawfish, contained unidentifiable fish remains some part of which might have been sockeye fry. Of the eight species, only the lake trout was confined to the lake. The others were taken both in the river and in the North Arm. Two predatory sculpins, *Cottus asper* and *C. aleuticus*, are known to inhabit the Babine drainage but neither was taken in the Upper River, where they are apparently not common.

Of the known predators, rainbow trout and juvenile coho were those most frequently captured in the Upper River. Many of the latter were smolts. On the basis of their apparent abundance and the coverage fry content of their stomachs, these two species would appear to be the most important predators on sockeye fry in the Upper River. The stomachs of several other species, the cutthroat, Dolly Varden, lake whitefish, and burbot, contained considerable numbers of fry but these predators seemed to be much less abundant than either rainbow trout or coho. The lake whitefish is ordinarily a bottom feeder and is morphologically ill-adapted for predation, an indication of the vulnerability of sockeye fry while in the river.

Rainbow trout and coho smolts were also the fish most frequently taken in the North Arm. However, they were less important as sockeye predators than in the Upper River. In fact, none of the rainbow trout taken in the lake contained an identifiable sockeye fry. There was also a decline in predation by cutthroat trout and lake whitefish. Although the proportion of burbot stomachs

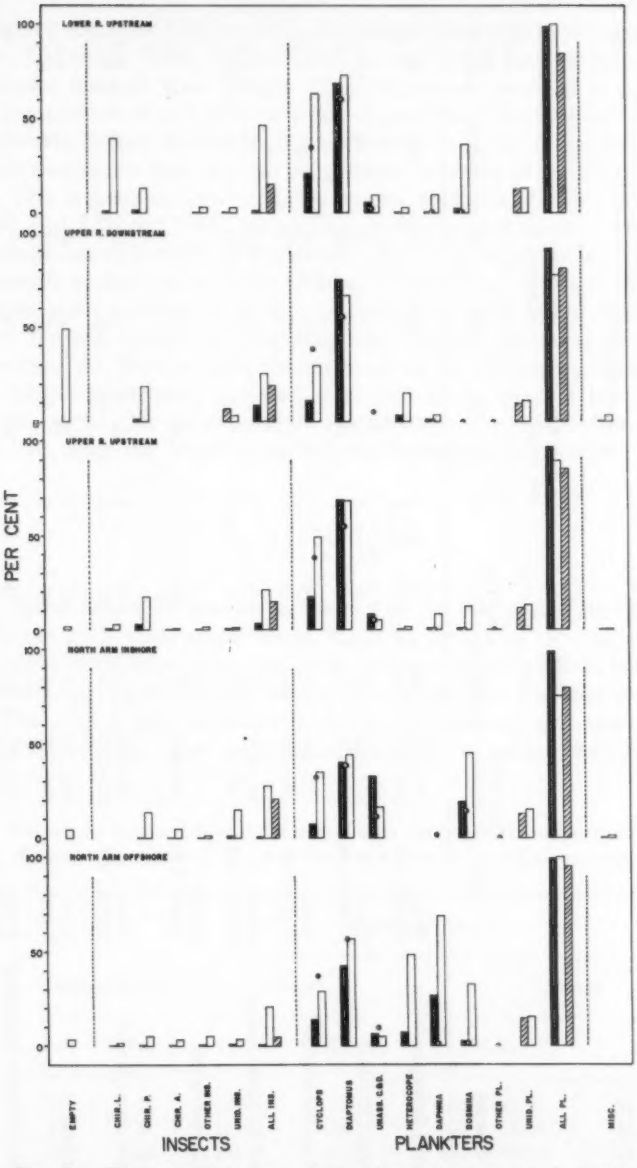

FIG. 21. The stomach contents of fry taken in five areas during 1965. Black bars, percentages of the total number of food organisms; white bars, percentages of the total number of stomach containing food; cross-hatched bars, estimated percentages of total volume of food. The circles indicate the percentages of each plankter in plankton samples from four areas. (The same plankton data are shown with both the upstream and downstream Upper River food data.) Further explanation in text.

TABLE IV. The stomach contents of predatory fishes captured in 1965 in the Upper Babine River and the North Arm of Babine Lake.

Species	Area	Size range (*mm*)	Stomachs		Stomachs with				Number of fry	
					Unidentified fish remains		Identified sockeye fry			
			Examined	Empty (%)	No.	%	No.	%	Total	Avg per stomach
Rainbow trout (*Salmo gairdneri*)	River	94–535	106	4	5	5.	24	23.	860	8.1
	North Arm	106–470	62	2	2	3.	0	0	0	0
Cutthroat trout (*S. clarki*)	River	212–362	9	0	1	11.	7	78.	94	10.4
	North Arm	124–313	12	8	2	17.	2	17.	6	.5
Coho (*Oncorhynchus kisutch*)	River	81–107	29	14	1	3.	20	70.	107	3.7
	North Arm	98–250	63	2	7	11.	6	10.	15	.2
Dolly Varden (*Salvelinus malma*)	River	198–207	2	0	0	0	2	100.	34	17.0
	North Arm	188	1	0	0	0	1	100.	10	10.0
Lake trout (*S. namaycush*)	River	–	0	–	–	–	–	–	–	–
	North Arm	183–458	23	30	7	30.	2	9.	7	.3
Lake whitefish (*Coregonus clupeaformis*)	River	388–505	12	8	4	33.	4	33.	63	5.3
	North Arm	214–380	6	33	0	0	0	0	0	0
Burbot (*Lota lota*)	River	441–737	5	0	1	20.	4	80.	20	4.0
	North Arm	286–610	8	38	0	0	2	25.	49	6.1
Squawfish (*Ptychocheilus oregonense*)	River	223–227	13	38	8	62.	0	0	0	0
	North Arm	183–385	23	52	3	25.	0	0	0	0

containing fry fell from 80% to 25%, the average number of fry per stomach increased. The single Dolly Varden taken in the North Arm contained 10 fry. Obviously, for most of these species, the samples are too small to permit any rigorous comparison of the relative rates of predation in the river and in the lake. However, it does appear that predation on sockeye fry is more intense in the river than in the lake: 6.7 fish per predator stomach in the river and 0.4 in the lake. This is presumably due to the greater availability of fry in the river.

Ward and Larkin (1964) found that in the western region of Shuswap Lake juvenile sockeye were an important food of rainbow trout. In years in which juvenile sockeye were scarce, fish as a food item fell below the average volume and the condition of trout was relatively poor. When sockeye were abundant, the fish content of stomachs rose and the condition of trout was above average. At Babine Lake there appear to be differences between river and lake in the availability of sockeye fry and in the average fry content of predator stomachs. The growth of 37 rainbow trout from the Upper Babine River and 31 from the North Arm was backcalculated from scales using the formula:

$$L_n = I + \frac{L_t - I}{S_t} S_n$$

where L_n is the length of the fish at the end of the nth year of life, I is the intercept of the body scale relationship, L_t is the length of the fish at capture, S_t is the length of the anterior radius of the scale at capture, and S_n is the length of the anterior radius of the scale within the nth annulus. I in this instance was 41 mm. The growth data (Table V and Fig. 22) indicate that trout from the river grow faster than those in the lake although the length–weight relation-

TABLE V. Calculated fork length (mm) at end of each year of life of rainbow trout from the Upper Babine River (U.R.) and the North Arm(N.A.) of Babine Lake.

	Number of fish		Calculated length			
			Mean		Range	
Age-group	U.R.	N.A.	U.R.	N.A.	U.R.	N.A.
I	37	31	75.0	71.3	61.5–92.6	56.8–93.6
II	37	31	115.1	108.5	90.0–140.2	85.1–146.1
III	37	22	194.0	170.8	123.6–245.4	118.5–212.0
IV	36	15	278.3	238.7	179.4–304.6	174.2–300.6
V	10	5	314.4	273.7	268.1–354.0	211.2–333.6
VI	2	0	418.7	–	401.4–436.0	–
VII	1	0	442.8	–	418.7	–

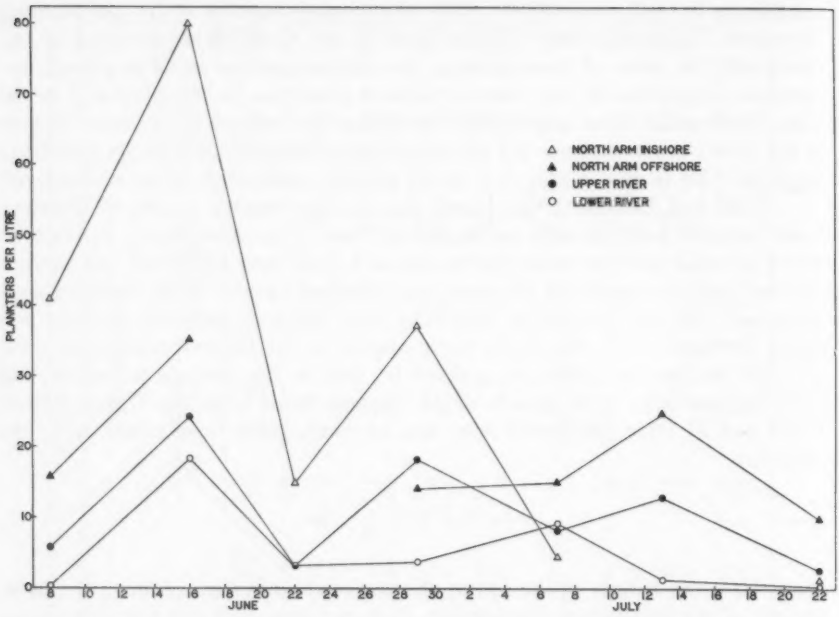

FIG. 22. The abundance of plankton in samples taken in various parts of the Babine River–North Arm area in 1965.

ships are similar (Fig. 22). The length–weight data are from 77 Upper River and 53 North Arm trout and are expressed as the mean weight of fish in each centimeter length class.

Possibly the difference in growth rates results from the greater availability of salmon eggs and salmon fry to the river trout. This hypothesis presumes that the two populations do not mix to any extent and that the two samples are representative of rainbow trout in their respective areas, two assumptions that cannot be justified at present.

Fish were not the only predators on fry in the rivers. The American merganser and Bonaparte gull were frequently observed in the river and in the area around Smokehouse Island, where they appeared to be feeding on sockeye fry. On several occasions the robin, *Turdus migratorius*, was seen pecking at fry at the cutbank in the Lower Babine River. The river at this point was particularly fast and many fry moved upstream within a few inches of the bank. The robin is certainly not a habitually aquatic feeder and its apparent success in capturing sockeye fry again emphasizes the latter's vulnerability to predation while in the river. Aside from these observations no detailed study was made of avian predation during 1964 and 1965. There is, however, some additional

information available on bird predation in the Babine River–Nilkitkwa Lake area during the 1953 and 1954 (V. I. F. Allen, unpublished report at the Biological Station, Nanaimo). This information is summarized in Table VI.

Except for Barrow's goldeneye, each of the species examined was found to be feeding occasionally on fish. In six species, these were highly identifible sockeye fry. Although the stomach analyses indicate a rather high fry consumption in some species, particularly some of the gulls, none was particularly abundant and Allen comments, " . . . it is believed that the information available shows that due to immense numbers of prey available, coupled with a limited bird predator population, it is possible that no serious bird predation problem exists." The author's own observations confirm this. Predation by fishes would appear to be a much more important factor in the mortality of sockeye fry in this area.

COMPARISON WITH FRY MOVEMENTS IN SOME OTHER AREAS

Upstream migrations of sockeye salmon fry occur widely in North America. Sizeable upstream movements have been observed in the Karluk River on Kodiak Island, Alaska, the Wannock River draining Owikeno Lake on the British Columbia coast (Foskett, 1958), the Chilko River in the Fraser system, the Little River below Shuswap Lake, B.C., as well as the Upper and Lower Babine rivers described herein. Each of these upstream fry migrations numbers many millions. Other, smaller upstream populations are known to occur. The upstream movements of sockeye fry in the Karluk and Chilko rivers have been investigated by the Fisheries Research Institute of the College of Fisheries, University of Washington, and the International Pacific Salmon Fisheries Commission respectively. Information relating to the Karluk migration was communicated by C. Walker, formerly of the Institute. The description of the Chilko run is taken from Andrew and Geen (1960) and personal communications from J. Roos of the Salmon Commission.

In general, the upstream migrations in both the Karluk and Chilko are very similar to that in the Babine rivers. There is an initial downstream movement of fry, a period of holding, and an eventual return upstream. In the Chilko, fry emerge at night and move downstream until they reach areas of low velocity along the river edge where they hold. Seven to 10 days elapse between the emergence of fry and their movement upstream. Fry moving upstream average several millimeters longer than downstream migrants.

Figure 23 illustrates the mean daily water temperatures and the seasonal periodicity of upstream movement in the Chilko River during 1958, 1959, and 1960, three representative years. Over the years, it has been observed that the upstream movement of fry does not begin in earnest until the mean daily water temperature exceeds 4 C and that in most years the run is over or in decline before the mean temperature exceeds 8 C. Ordinarily, water temperatures increase gradually from late April through June with relatively little day-to-day temperature change. Rarely do temperatures fluctuate over 1 C from one day

TABLE VI. Stomach contents of birds collected by I. V. F. Allen in the Babine River–Nilkitkwa Lake area in 1953 and 1954 with some census information indicating the abundance of each species within the area. The census data indicate first, the maximum number of birds seen on any single day and second, the mean number per day for several censuses made during the months of May, June, and July each year.

Species	No. stomachs examined	No. empty	No. with fish	Stomachs with sockeye fry (%)	Total fry	Average fry per stomach	Census results May–July			
							1953		1954	
							Max.	Mean	Max.	Mean
Common loon (*Gavia immer*)	1	0	1	0	0	0	5	3.3	5	3.0
Red-necked grebe (*Colymbus grisegena*)	7	1	1	0	0	0	6	4.0	14	–
Barrow's goldeneye (*Glaucionetta islandica*)	4	0	0	0	0	0	11	4.4	–	–
American merganser (*Mergus merganser*)	8	1	7	25	6	.7	14	7.0	10	6.4
Herring gull (*Largus argentatus*)	5	3	1	0	0	0	2	1.2	3	2.4
Ring-billed gull (*Larus delawarensis*)	2	0	2	100	19	9.5	1	1.0	5	4.0
Short-billed gull (*Larus canus*)	7	0	5	43	145	20.7	3	1.7	3	2.6
Bonaparte gull (*Larus philadelphia*)	10	1	4	20	78	7.8	16	5.3	27	16.8
Black tern (*Chilidonias nigra*)	6	0	4	17	11	1.8	16	8.3	13	7.3
Belted kingfisher (*Megaceryle alcyon*)	3	0	3	0	0	0	5	1.8	2	1.6
Harlequin duck (*Histrionicus histrionicus*)	2	0	1	50	6	3.0	2	1.5	2	1.7

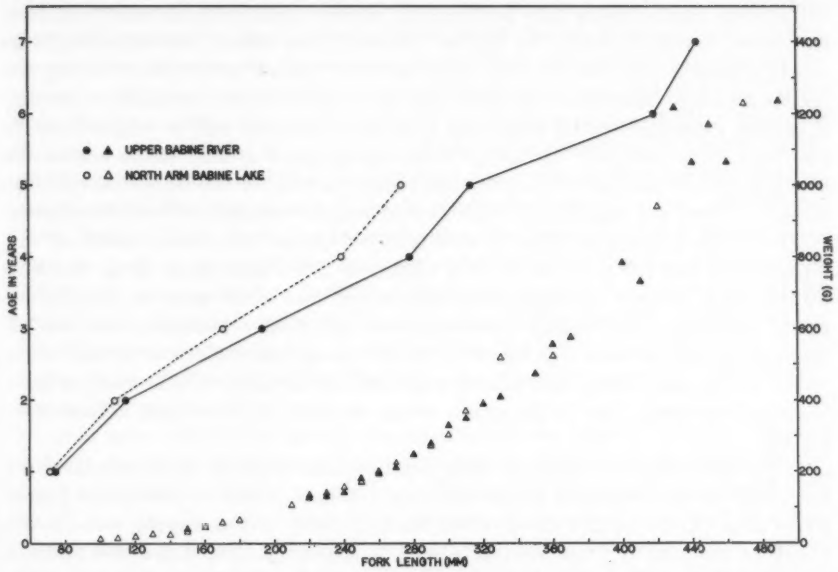

FIG. 23. Length–weight relationships (triangles) and calculated growth (circles) of rainbow trout taken in the Upper Babine River and the North Arm of Babine Lake 1965.

to the next. Small temperature surges do occur, however, and in some years, for instance 1960, there is evidence of a correspondence between water temperature peaks and upstream movement. Aside from such relatively minor fluctuations, the periodicity of upstream movement is broadly peaked and unimodal. Upstream movement generally occurs over about 50 days, from May to mid- or late June. In 1958, when water temperatures were unseasonably high, the upstream migration began April 27 and ended before June 1.

As in the Babine rivers, the upstream movement of fry in the Chilko River appears to be almost entirely confined to the river bank. Andrews and Geen (1960) describe the fry as moving upstream in shallow water not more than 0.46 m from shore at an average rate of 7.9 cm/sec against average water velocities of about 16.5 cm/sec.

The diel pattern of upstream movement in the Chilko is characterized by a sharp peak of activity at about 0500 hr, a mid-morning depression, and a period of moderate activity through the mid- and late afternoon. Fry are active from about 0300 hr to 2300 hr. This pattern is similar in many respects to that of fry in the Upper Babine River. One feature not observed at Babine is the occasional movement downstream along the banks of large numbers of fry known to have recently migrated upstream. The reason for this is unknown.

Fry emergence in the Karluk River is also largely nocturnal. After emergence, fry move downstream some distance before becoming associated with

the stream banks where they accumulate in pool areas. During May and June fry move upstream along the banks. These fry are about the same length as newly emerged fry. During this early summer period, traps placed along the banks capture upstream migrants day and night. There is usually a second upstream movement of fry from late July to at least the end of August and, in one year, into October. These fry could be trapped at any point across the width of the stream and were not readily seen. In neither the Babine nor Chilko Rivers is there any significant offshore upstream movement of fry. The traps at Karluk were suspended from a weir which blocked the entire width of the stream and the fry may have been captured while searching along the weir for an easy passage through. Such behaviour was often seen in the Babine River wherever the passage upstream was practically blocked. The Karluk late-run fry had a mean fork length of 50–55 mm in comparison with the 31 mm of the earlier run. These late season migrants are thought to be fry which have remained for some time in the quiet, weedy sections of the stream before moving up.

An interesting variation in upstream migrations occurs in Morris Creek, a tributary of the Harrison River draining Harrison Lake in the lower Fraser River area (Andrews and Geen, 1960: Ricker, 1960). Fry emerging into Morris Creek or entering it from Weaver Creek, a tributary, first move downstream into the Harrison River. Some apparently remain in the sloughs bordering the Harrison River but others move upstream into Harrison Lake where they remain until smolting.

The upstream movement in the Little River (Andrews and Geen, 1960) is even more complex. Fry emerging in the Little River are borne downstream into Little Shuswap Lake where they remain for several weeks before reentering the river and migrating upstream into Shuswap Lake. Fry from the South Thompson River, below Little Shuswap Lake, pass upstream into Little Shuswap Lake and may continue upstream through the Little River into Shuswap Lake. In addition, there are indications that fry moving downstream from the Adams River, which enters Shuswap Lake near where the Little River flows out, are occasionally swept down into the Little River and must then move upstream to reenter Shuswap Lake. Fry in the Little River, which have lingered for some time in Little Shuswap Lake and are larger at the time of upstream movement than Chilko River fish, have been observed as much as 3 m offshore in water velocities of 30–60 cm/sec.

Some mention should be made of certain streams in which sockeye fry are known to hold along the stream edges for a time although upstream movement apparently does not occur and the fry eventually move downstream into lakes. Hartman et al. (1962) describe fry holding along the edges of Hidden Creek close to where the stream enters Brooks Lake. None of these remained more than a single day and all of them moved on downstream at nightfall to be replaced at dawn. Fry are known to frequent the stream edges for longer periods of time in the Brooks River between Brooks Lake and Naknek Lake (W. Heard, personal communication). Passage upstream into Brooks Lake is

blocked by a small falls (not impassable to adult sockeye) and the fry ultimately move downstream. In the Agulowak River in the Woods River system, Alaska, another river between two lakes, sockeye fry often remain for long periods before eventually moving downstream, some 50 mm or larger (Burgner and Green, 1963). Burgner (1962) has also described fry in some of the smaller spawning tributaries of the Wood River system which attain considerable growth before moving downstream. In other downstream situations such behaviour has not been observed. For instance (A. Coburn, personal communication), at Williams Creek, Lakelse Lake, B. C., an intensive search of several miles of stream bank failed to turn up a single fry during the height of the spring outmigration. Holding fry are equally rare in Scully Creek, another tributary of the same lake. In the Fulton River no more than a few isolated individuals have been holding along the stream edges during the day, either upriver or in slower parts of the stream close to where it enters Babine Lake (G. Wilson and J. MacDonald, personal communication).

It is very difficult to identify features which are typical of rivers supporting large upstream migrations. The water may be clear as in the Upper Babine River or somewhat turbid as it is in the Wannock River draining glacial Owikeno Lake. Holding areas may be abundant (Upper Babine River) or sparse (Lower Babine River). Water temperatures during the migration may be relatively high with large day-to-day fluctuations (the Upper Babine River) or low with little day-to-day variability (Chilko). The migration itself may be seasonally very early (Chilko) or very late (the Upper Babine River and the Karluk late run). The most important environmental factor limiting upstream migration would appear to be water velocity. None of the streams with a large upstream migration is particularly swift and where high velocity sections occur, fry seem to experience great difficulty. Andrews and Geen (1960) state, ". . . in any river where sockeye fry are required to migrate upstream to their rearing lake, continuous marginal paths in which the velocity does not exceed 0.5 fps (15.2 cm/sec) for a distance of 1.5 ft (46 cm) should be available." These represent ideal conditions and fry have been observed, in the Babine Rivers and in other areas, passing through short stretches of water in which water velocities considerably exceeded this. However, there is little doubt that prolonged exposure to high velocities would severely limit upstream movement.

COMPARISON WITH FRY MOVEMENTS IN THE FULTON RIVER

The Fulton River flows from Fulton Lake into the main body of Babine Lake (Fig. 1). The upstream movement of spawning sockeye is blocked by an impassable falls immediately below Fulton Lake and the entire fry population of the river moves downstream into Babine Lake. The Resource Development Branch of the Department of Fisheries of Canada has studied the sockeye population of this stream since the fall of 1961. There is thus a considerable body of information available for comparison with what is known of sockeye salmon in the Upper Babine River.

In the Fulton River, the spawning of sockeye salmon occurs over a 6–8 week period from about September 1 to the latter part of October. Spawning ordinarily peaks during the last week in September but may be delayed, as it was in 1963, when water temperatures remained unseasonably high. In the Upper Babine River the spawning period is the latest in the Babine system extending from about October 1 to the last week in November and occasionally into December. Spawning in this river peaks about October 20. The periods of fry emergence in the two rivers correspond in their order to that of spawning: in the Fulton River from May to mid-June with a peak during the last week in May; in the Upper River from early May to at least mid-July with the midpoint about June 15.

As previously indicated, the seasonal periodicity of downstream sockeye fry movements in the Fulton River is closely related to spring discharge patterns. Figure 7 illustrates the seasonal periodicity in 1964 only, but the pattern is typical of that during 1962, 1963, and 1965. In each of these years the downstream run reached its peak during a period of high discharge, declining precipitously thereafter. In the Upper Babine River, water temperatures seem to have the greatest influence on seasonal patterns of emergence and downstream movement.

Northcote (1962) found that water temperature was the single most important factor determining the current responses of juvenile rainbow trout. These fish were able to hold position and move upstream only where water temperatures were relatively high (daily maximum usually 15 C). Northcote (1962) demonstrated first, that there were consistent differences in the seasonal temperature regimes of streams inhabited by upstream and downstream populations and, second, that these were of sufficient magnitude to affect the behaviour of fry. Figure 24 compares the seasonal temperature regimes of the Fulton River and the Upper Babine River during the spring and early summer of 1964 and 1965. The durations and approximate midpoints of the downstream fry runs in the two rivers are indicated.

The most obvious difference in the seasonal temperature patterns of the two streams is the contrast between the smoothly increasing water temperatures of the Fulton River and the fluctuating temperature of the Upper Babine River. However, 'though water temperatures in the Upper Babine River exceeded those in the Fulton River during peaks, the mean temperature of the Fulton River during the period May 1 to July 15, 1964 was higher than that of the Upper River. The 1965 data do not extend beyond June 21 but a similar pattern was emerging. A simple comparison of seasonal temperature regimes is, however, not entirely satisfactory because of differences in the timing of emergence and downstream movement in the two rivers. In both 1964 and 1965, Fulton River fry emerged and moved downstream in appreciable numbers earlier in the season and at lower temperatures than Upper River fry. If water temperature were a major factor in determining the current responses of sockeye fry in the Fulton River it should be demonstrable that the temperatures prevailing during the period of fry emergence were so low as to preclude holding and upstream movement. The evidence suggests that this is not so.

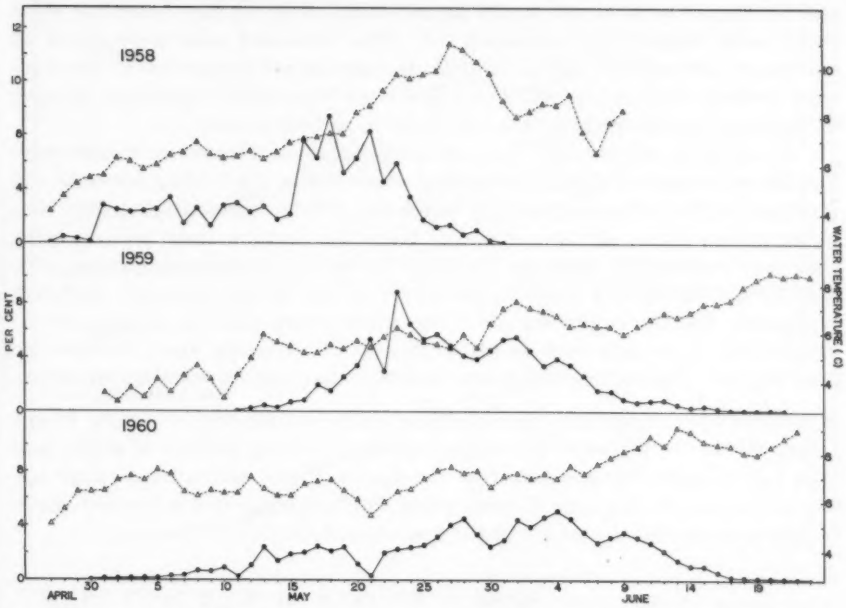

FIG. 24. The seasonal temperature regimes (triangles) and the seasonal periodicities of upstream fry movements (circles) in the Chilko River, 1958 through 1960. Data supplied by J. Roos of the International Pacific Salmon Fisheries Commission.

In the Upper and Lower Babine rivers fry have been observed holding position throughout the night at water temperatures as low as 5 C. Active upstream migrations occurred at the same temperatures. In the Chilko River, major daily upstream runs occur at temperatures of 4 C. Under experimental conditions, the current response of sockeye fry has been found to be remarkably consistent over a wide range of temperatures. Keenleyside and Hoar (1954) observed a marked, positive rheotaxis over a temperature range of 4 to 24 C. Brett et al. (1958) found that fry would orient to, and swim with, a moving background at temperatures from 1 to 24 C, almost the entire range of temerature tolerance of the species.

For purposes of argument, 8 C would seem to be a temperature at which most sockeye fry could either maintain position along the stream edge or move actively upstream. In the Upper Babine River large numbers of fish have been observed holding and moving upstream at temperatures less than this and in the Chilko River, in some years, mean water temperatures during the upstream fry migration never reach 8 C. At this temperature, the maximum sustainable cruising speed of juvenile sockeye is approximately 75% of the maximum sustainable effort at any temperature (Brett et al., 1958). An analysis of the Fulton River fry catches for 1964 and 1965 reveals that respectively 28.1%

and 59.9% of the total fry output passed downstream on days when the mean daily water temperature exceeded 8 C. The estimated runs were about 39 million in 1964 and 25 million in 1965. If a significant proportion of these fry were holding along the banks they would have been readily apparent. In spite of this, none were seen and very few could have been present.

It might be argued that it is not simply the absolute water temperature but the occurrence of sharp fluctuations which effects the holding response and upstream migration in sockeye fry. Northcote (1962) suggest that sudden rises in temperature may act as a directing factor for rainbow trout fry, orienting their movements in an upstream direction. If this kind of mechanism is operative then we should expect a much higher degree of holding and upstream migration in streams like the Upper Babine River where sharp rises in temperature do occur, than in streams such as the Fulton in which temperature changes are more regular. The evidence suggests, however, that large upstream movements of sockeye fry can occur in the absence of extreme temperature surges. In the Upper River, for instance, fry moved upstream during periods of stable and even falling water temperatures. In the Chilko River, which has a large upstream movement, there are in many years, only relatively minor fluctuations in temperature during the course of the upstream migration.

GENERAL DISCUSSION

The published descriptions (Hoar, 1958; McDonald, 1960; Hartman et al., 1962; Heard, 1965) of the behaviour of sockeye salmon fry during emergence and downstream migration can be summarized as follows. (1) The fry are negatively phototactic and during the day most remain either buried in the gravel or hidden in shaded holding areas along the stream banks; (2) with the coming of darkness those fry which are ready to migrate emerge from their hiding places and exhibit a marked negative rheotaxis, swimming downstream; (3) downstream migrants act as individuals while migrating and do not school. As a result of this pattern of behaviour, newly emerged fry very rapidly leave the spawning stream and enter the lake nursery. In most instances, the journey is probably completed in a single night.

Once in the lake, the behaviour of juvenile sockeye salmon changes radically. The fish abandon their previously solitary habits and form schools. Fry in schools show less cover response and remain, swimming and feeding, in brightly lit areas. They are positively rheotactic and are able to maintain position in strong currents (Keenleyside and Hoar, 1954).

Hoar (1958) suggests that these changes in the behaviour of older fry, coupled with an increase in size and swimming ability, might explain upstream migration. The behaviour of upstream migrants in the Babine rivers is, in fact, very similar to that of downstream migrants after they enter lakes: they form schools, they move and feed in the open, they react positively to current and hold position during the night. In addition, fry moving upstream are generally larger than those moving down. However, it is difficult to see how fry emerging

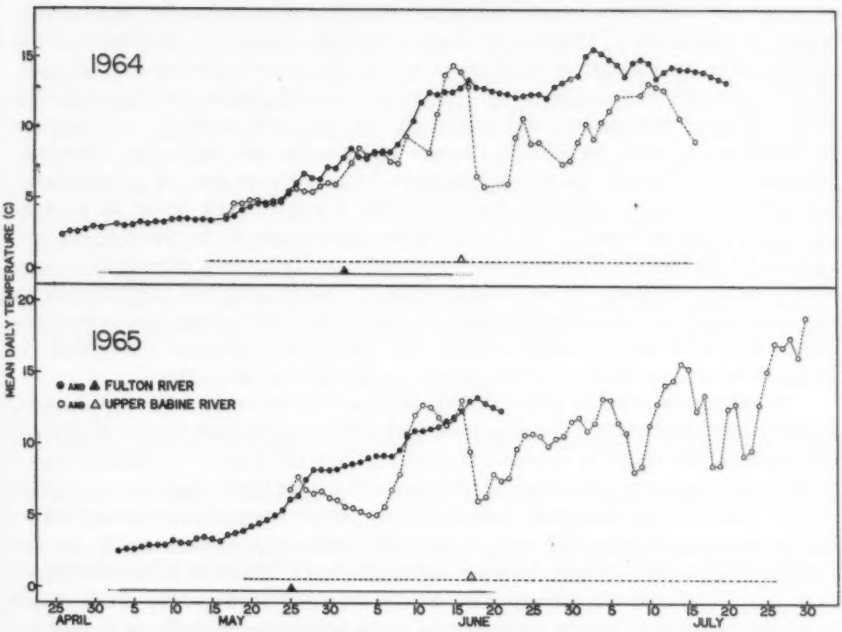

FIG. 25. Mean daily water temperatures of the Upper Babine and Fulton Rivers during the period of fry movements in 1964 and 1965 with the duration and approximate midpoints of emergence and downstream movements.

in a situation such as that in the Lower Babine River could survive unless they were immediately possessed of these behavioural characteristics. If, as Hoar (1958) suggests, they were subject to progressive development and if fry were borne downstream before these characteristics appeared, catastrophic mortalities would occur. In the Lower Babine River the major spawning beds are only a short distance above the adult enumeration weir, an easy night's journey even if fry were being borne passively downstream. Some fry are swept through the weir but millions more reach the river banks and survive to move upstream into Nilkitkwa Lake. The Babine River studies support Hartman et al. (1962) who, commenting on the upstream movement of sockeye fry in the Karluk River, states, "Apparently schooling and the adaptive changes to positive rheotaxis and loss of negative rheotaxis associated with it take place before the emerging fry reach the sea, or perhaps before they move very far from the redd sites." There is apparently a precocious development of behavioural responses which, in fry from typical downstream situations, are acquired only after the fry have left the stream and entered the lake.

Northcote (1962), in his study of the migratory behaviour of juvenile rainbow trout at Loon Lake, B.C., concluded that the differential responses

of upstream and downstream migrants to current were primarily regulated by water temperature. Temperature had a twofold action: it set limits within which upstream migration could occur and it affected the timing and intensity of the responses when it did occur. There is no doubt that in the Upper Babine River water temperatures did affect the timing and intensity of upstream movement, at least seasonally though apparently not diurnally. However, there is no indication that water temperatures were so low as to absolutely preclude holding or upstream migration and fry were seen doing so at temperatures ranging from 5 C to 17 C. During the course of the downstream migration in the Fulton River, water temperatures frequently exceeded those at which large numbers of fry are able to reach the banks and hold position or even move upstream in other systems and yet this type of behaviour has never been observed. From this it would appear that water temperature differences, in themselves, do not determine the ultimate direction of migration.

Northcote was led to reject the hypothesis that there were "... genetically distinct inlet and outlet stocks, each maintaining appropriate innate behaviour responses which result in movement of young into the lake ...." In the case of sockeye fry, however, this does appear to be the most likely explanation. There are no consistently observed differences in water temperature or any other environmental factor which will explain the observed differences in the behaviour of downstream and upstream sockeye stocks. There is, in fact, evidence that under experimental conditions newly emerged sockeye fry from outlet populations have a greater tendency to move upstream than those from inlet populations (E. Brannon, personal communication).

The existence of an intermediate type of fry behaviour in which fry hold for a time along the stream edge but eventually move downstream has been noted. Possibly the development of upstream migratory habits involves selection for behavioural tendencies which exist to some degree in all stream-spawning sockeye populations.

In what situations are outlet spawning and the upstream fry migration which it entails likely to develop? In the first place an upstream migration must be a physical possibility. This appears to be primarily a question of water velocity. Second, and possibly most important, at the termination of their upstream movement the fry must enter a favourable lacustrine environment. The mortality to fry between the time of emergence and the time that they take up lake residence must be very much greater in upstream than in downstream migratory stocks due to downstream loss, to intensive predation, and, possibly, to the effects of intraspecific competition for food during the time when the fry are confined to a very limited area along the stream edge. These severe mortalities must be balanced at some period of the life cycle. Outlet streams are generally more stable than inlet streams and this probably enhances the egg-to-fry survival of outlet stocks. Even so, in highly competitive lacustrine environments already adequately supplied by spawning in inlet streams, the particular behaviour patterns which facilitate upstream migration may be at a selective disadvantage.

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NOTES

Lethal Endrin Concentration in the Blood of Gizzard Shad

One of the persistent problems facing pollution biologists is the identification of the materials that cause fish kills throughout the country. These situations are especially troublesome in areas of concentrated populations and/or industries that compound the possible sources of pollution. In many cases the actual cause is a slug release of a toxicant that has been diluted and passed beyond the location where dead fish and water samples would be collected at least several hours after mortality occurred. Autopsy methods based on analyses of fish tissues can provide the information necessary to identify the specific toxicant. Previous techniques for zinc-caused fish mortality (Mount, 1964) and endrin toxicity to channel catfish (Mount et al., 1966) have been established at the Newtown Laboratory, Federal Water Pollution Control Administration.

In the studies being reported, additional information was sought on autopsy techniques and the feasibility of using gizzard shad, *Dorosoma cepedianum* (LeSueur), as a test species was explored. This species has an extensive range and is very common in the eastern half of the United States. It is also frequently involved in fish kills, apparently being relatively more sensitive than other species to many toxicants. Irwin (1965) determined that of 57 species of fish, gizzard shad is the least resistant to petroleum refinery effluent, and he considered them to be undesirable as a test animal.

Adult gizzard shad, 8–12 inches in length, were collected in April 1965 from Rocky Fork Lake in southern Ohio by means of a fish trap utilized by the Ohio Division of Wildlife for fish population estimates. The shad were in these traps for up to 24 hr before being placed in holding tanks for the 50-mile trip to the laboratory. These fish were observed and treated with antibiotic for 2 weeks before experimentation began. During this time and the additional 2 weeks of exposures, mortality due to causes other than endrin was less than 10%.

The test water was carbon-filtered tap water that was held in a 100,000-gallon outdoor concrete reservoir for about 2 months before use. This water, maintained at about 16 C, was passed to a constant-head box connected to a serial dilution apparatus (Mount and Warner, 1965) that established five endrin concentrations and a control. The chemical characteristics of this test water were 5.4 mg/liter dissolved oxygen, *p*H of 7.5, alkalinity of 45 mg/liter as $CaCO_3$, acidity of 3 mg/liter as $CaCO_3$, and hardness of 128 mg/liter as $CaCO_3$. Results were obtained by previously described procedures (American Public Health Association, 1960).

A total of 31 blood samples from 39 laboratory-exposed fish were analyzed for endrin. When more than one fish died in a test chamber and there was in-

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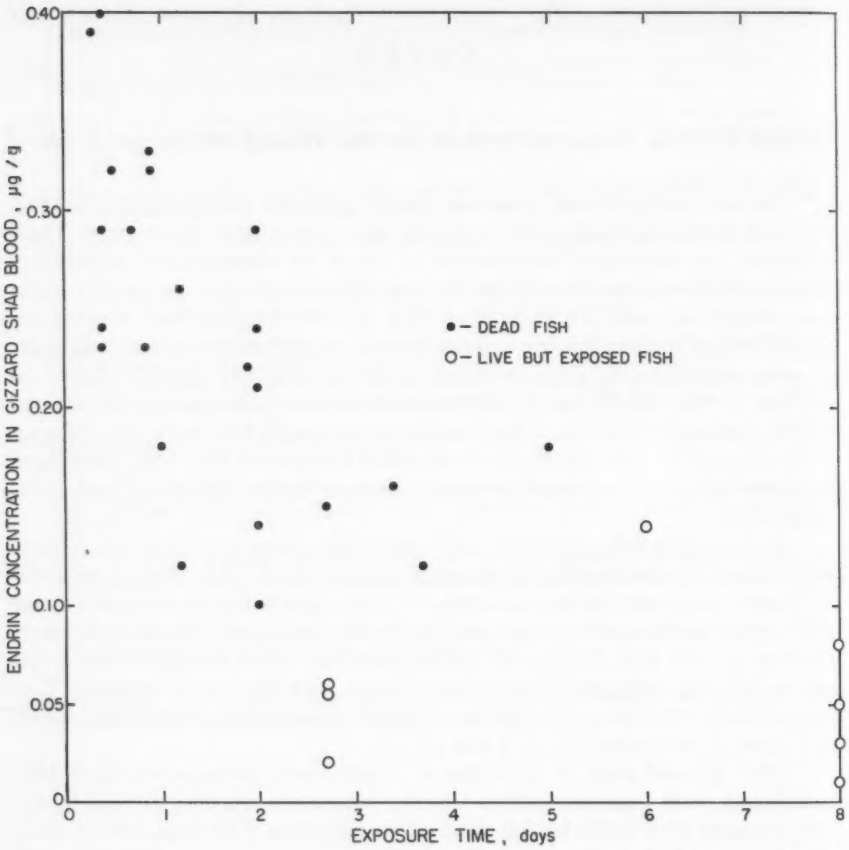

FIG. 1. Endrin concentrations in the blood of living and dead gizzard shad exposed to endrin in the laboratory.

TABLE I. Concentrations of endrin in blood of gizzard shad.

Condition and source	Number of samples	Number of fish	Mean endrin concentrations (µg/g)	Range (µg/g)
Dead, laboratory-exposed	23	29	0.24	0.10–0.40
Dead, field-exposed[a]	2	20	0.19	0.15–0.22
Live, laboratory-exposed	8	10	0.06	0.01–0.14
Live, field-exposed[b]	10	10	0.03	0.01–0.13

[a]Collected from Mississippi and Atchafalaya Rivers, 1963–64.
[b]Collected from same general locations, Jan.–Feb. 1965.

sufficient blood for individual analyses, the samples were composited. In addition, eight control shad were bled to determine baseline levels of endrin in the blood. Blood samples fortified with endrin were interspersed with the experimental samples to determine the accuracy of the method; the average of seven recoveries was 93%. All blood samples were assayed by gas chromatography using the procedure described by Mount et al. (1966). After these analyses, thin-layer chromatographic techniques (Smith and Eichelberger, 1965) were used for one-third of the experimental samples and the presence of endrin was verified in each. Concentrations of endrin in the blood of these laboratory-exposed fish were compared with blood concentrations found in shad killed during the fish kill in the Mississippi and Atchafalaya Rivers in 1963–64 and in living shad collected from the same area 1 year later.

Endrin concentrations in the blood of laboratory-exposed living and killed shad are related to time of exposure (Fig. 1). Means of raw data are listed in Table I. Endrin in the eight blood samples from control shad did not exceed 0.01 μg/g of whole blood. Dieldrin was present in the control fish blood at concentrations of less than 0.02 μg/g. Only blood from dead fish had endrin levels higher than 0.14 μg/g (average = 0.24), and only blood from live fish had endrin concentrations less than 0.10 μg/g (average = 0.06). These results indicate a critical level of endrin in the blood of 0.10 μg/g above which very few gizzard shad could survive (in this study only 1 of 24 survived). The critical level for channel catfish (Mount et al., 1966) was 0.30 μg/g.

Results of analyses of blood from field-exposed gizzard shad are also included in Table I. Two composite blood samples, taken from 20 dead or dying shad during the fish kill in the Mississippi and Atchafalaya Rivers in 1963–64, had endrin levels of 0.15 and 0.22 μg/g (Anon., 1964). These two samples were assayed by the method of Schafer et al. (1963). Ten blood samples taken from live shad collected in the same area 1 year later had endrin concentrations of from 0.01 to 0.13 μg/g. The latter samples were assayed by the same method (Mount et al., 1966) used for the laboratory samples. Only one of these 12 samples (0.13 μg/g) did not conform with the aforementioned critical level of endrin (0.10 μg/g) in the blood of gizzard shad.

The critical level of endrin in the blood (0.10 μg/g) of gizzard shad obtained as a result of laboratory exposures is also applicable for shad exposed to endrin in a natural water system. These data indicate that the critical level of endrin in the blood (the level above which few shad can survive) can be used for the detection of endrin-caused mortality.

The authors are grateful to Mr Charles E. Stephan and his staff who prepared and analyzed most of the gizzard shad blood samples.

Federal Water Pollution Control Administration
U.S. Department of the Interior, Cincinnati, Ohio

W. A. Brungs
D. I. Mount

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Scale Regeneration in the Bluespot Goby, *Coryphopterus nicholsi*

The bluespot goby, *Coryphopterus nicholsi*, is a common resident of subtidal waters in the Gulf of Georgia, British Columbia. It is most numerous where loose rock and boulders provide an abundance of hiding places. During the summer of 1963, a number of large collections were made in the Malaspina Strait–Jervis Inlet area using rotenone and scuba gear. Fish from one of these collections, taken June 24, 1963, are the subject of the following discussion. These are now in the Museum of the Institute of Fisheries at the University of British Columbia (Catalogue No. BC 63-934).

A comparison was made of regeneration in scales from two areas of the body: the area immediately above the lateral line and the area under the pectoral fin. Two samples of fish were chosen both of which included a wide range of sizes. From one sample of 31 fish, all available scales were removed from the first scale row above the lateral line on the fish's left side, an average of approximately 18 scales. In the other, smaller sample, 10 scales were removed from

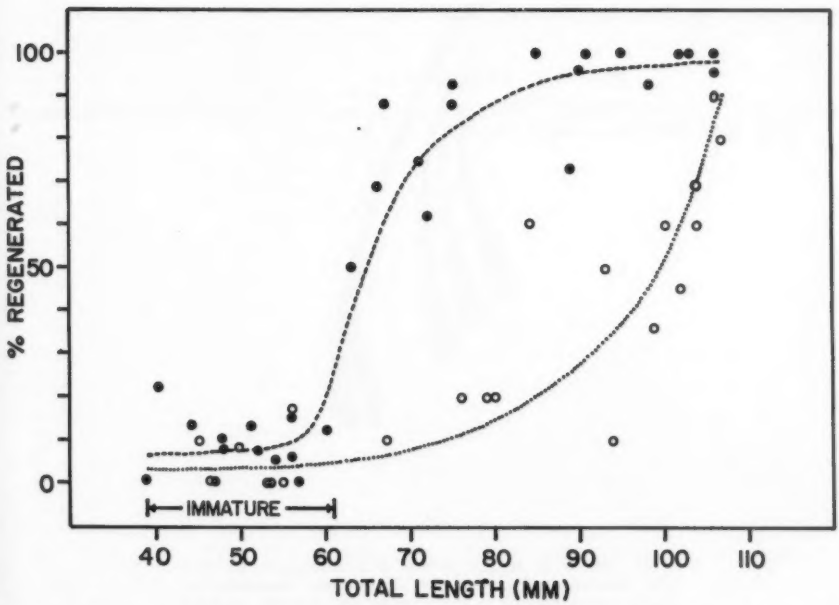

FIG. 1. Percentages of regenerated scales in samples taken from the first scale row above the lateral line (black circles and broken line) and from under the pectoral fin (white circles and dotted line) in *C. nicholsi*. The trend lines were fitted by eye.

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under the left pectoral fin of each of 20 fish. The scales were sandwiched dry between two glass microscope slides and examined at a magnification of ×50. The total length and state of maturity of each specimen were recorded. The results are illustrated in Fig. 1.

In both areas of the body the percentage of regenerated scales was very low in immature fish. However, the incidence of regeneration in scales from above the lateral line increased rapidly in fish between 60 and 80 mm and exceeded 90% in most fish larger than 80 mm. The incidence remained low in scales from under the pectoral fin until the fish had attained a length of 80 to 90 mm when there was a sharp increase.

The reasons for the high incidence of regenerated scales are not definitely known but the apparent coincidence of maturation and the sharp increase in the proportion of regenerated scales above the lateral line is suggestive. These fish are known to have a fairly elaborate courtship and nesting behaviour (Ebert and Turner, 1962) and this sudden increase may be largely the result of mechanical loss during the mating period. Scales under the pectoral fin, better protected from loss through abrasion, would be retained longer.

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

P. McCart

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Moult in the Pacific Harbour Seal *Phoca vitulina richardi*

The Pacific harbour seal, *Phoca vitulina richardi* (Gray), has become a commercial species and the basis of a fishery in British Columbia and adjacent coastal areas. Seal skins which are obviously not "prime" bring low prices and those which are actively shedding are rejected by fur buyers. An analysis of the moult process and a determination of the annual periods when moult could be expected were required for the information of the hunter and for use in consideration of regulations for protection of the fishery.

The harbour seal possesses a complete mammalian hair system (less the erector pili muscle) consisting of guard hairs, underhairs, sebaceous and sweat glands (Montagna and Harrison, 1957; Sokolov, 1960). Hair groups contain one large bristle-like overhair and one to six shorter, finer underhairs all of which emerge from a single surface opening (Fig. 1). The overhair follicle slants posteriad from the bulb to the surface of the skin. Underhair follicles are located adjacent and posterior to the guard hair follicle (Montagna and Harrison, 1957; Sokolov, 1960; Scheffer, 1964).

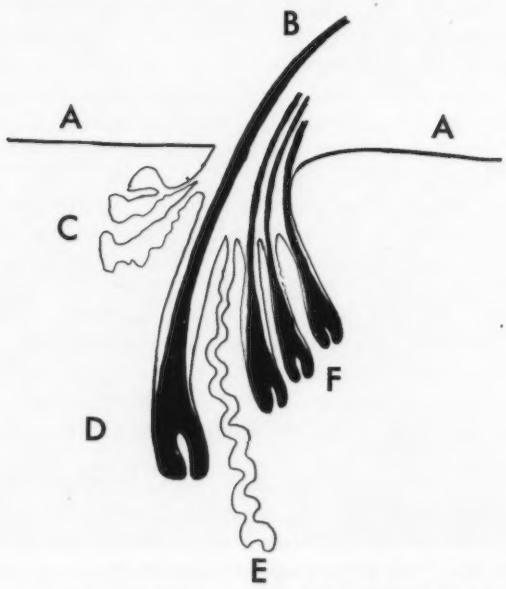

FIG. 1. Diagram of a follicle group in lateral view. A, epidermal surface; B, overhair and underhairs protruding through the pilary orifice; C, sebaceous gland, much simplified; D, overhair bulb and follicle; E, sweat gland, much simplified; F, underhair bulbs and follicles; (after Montagna and Harrisson, 1957; King, 1964).

J. FISH. RES. BD. CANADA, 24(2), 1967.
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In *P. vitulina* the stages of the hair cycle are similar to those described for mammals of other orders (Montagna and Harrison, 1957; Montagna and Van Scott, 1958; Bullough and Laurence, 1958). The quiescent follicle of telogen, the resting or "club hair" phase of the hair cycle, develops into the active hair-producing structure of anagen. When hair ceases to grow, the highly developed hair bulbs and dermal papillae of anagen quickly regress in catagen, or regression phase. The club is then established and the follicle enters telogen and remains quiescent until the annual cycle begins anew (Bullough and Laurence, 1958) unless the hair is pulled out (Montagna and Harrison, 1957).

The principal sources of skin samples were a commercial fur buyer's plant where a very large number of skins from all areas between western Alaska and Washington State were made available for study, and a skin collection from southern British Columbia and Puget Sound which was accompanied by the lower jaws of the specimens. Accurate aging of the second group was accomplished by Mr Michael Bigg, using the method outlined by Mansfield and Fisher (1960).

Skin samples for histological study were sectioned at 8 μ, mounted in serial sections, and stained with haematoxylin and eosin. Samples from 261 skins, randomly but unevenly distributed across the year, were analyzed microscopically to determine the stage of follicle development of each individual and the percentage of specimens in each stage of the moult cycle for each month.

Personal scrutiny and photography of seal pelts and carcasses provided the basis for descriptions of patterns of moult progression in the harbour seal.

References in the literature indicate that an annual moult occurs in *P. vitulina* as early as June (Murie, 1959) and as late as October (Scheffer and Slipp, 1944).

External signs of moult such as fading pelage, brittle hair, and shedding hair are noted over much of the year on the British Columbia coast and adjoining regions. "Shedder" skins collected at Anacortes, Washington, on January 10, 1965 (H. Hansen, personal communication) and a moulting adult male at Howe Sound, B.C., on November 21, 1965 (Bigg, personal communication) are the earliest and latest moult dates reported. Unexpected anomalies have been reported such as a population of seals at Smith Island, Washington, which provided prime post-moult pelts in October when the seals of nearby Anacortes were reported to be "100% shedders" (March and H. Hansen, personal communication).

Degeneration of hair from prime condition has become noticeable in some skins by February. Hair on ventral areas begins to turn pale and begins to feel coarser than hair on the dorsum. Black hairs on the dorsal surface develop brown or whitish tips. The white rings of characteristic spot patterns become pale tan and the black centers of rosettes begin to brown. As the process continues, bare or worn areas may appear in the "armpits" at the bases of the anterior flippers. Actual moult may take place in almost any month of the year. The results of microscopic analysis (Table I) indicated that anagen occurred as early as February, involved most of the population in July–September and

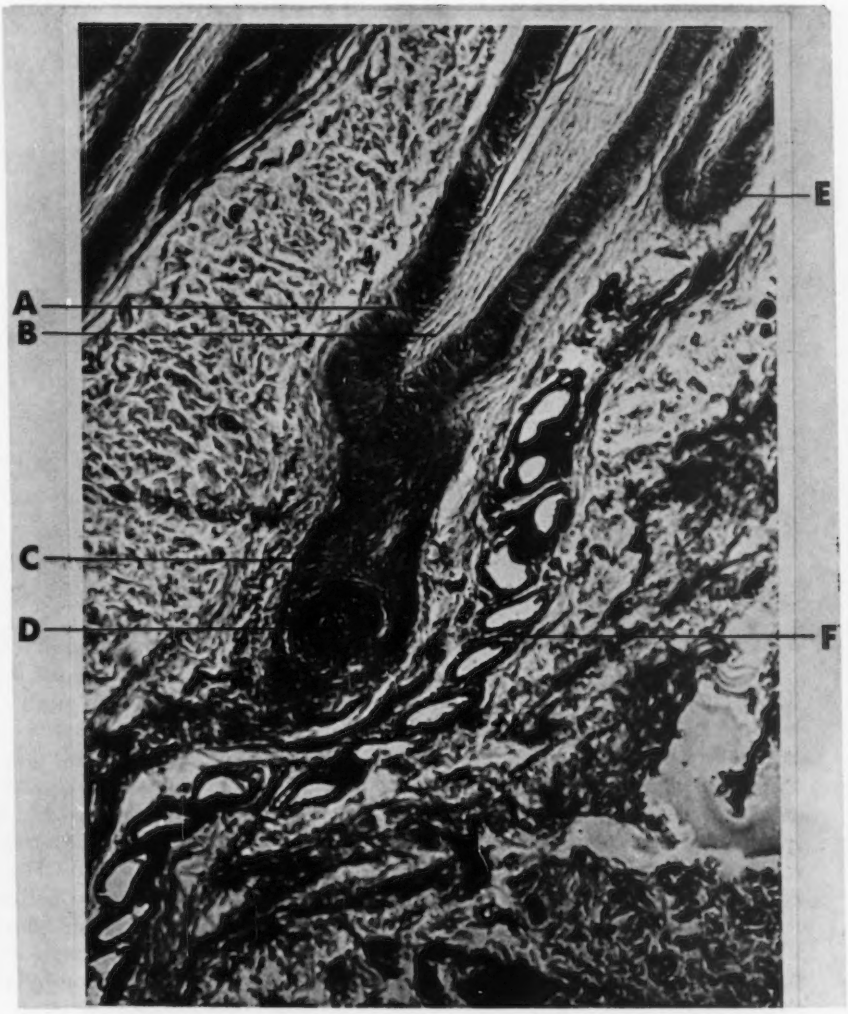

FIG. 2. The hair follicle in telogen. A, follicle of overhair; B, club hair; C, bulb; D, papilla remnant; E, underhair follicle; F, sweat gland: sections cut through convolutions.

TABLE I. Percentages of moult progression by months.

	Male	Female	Totals[a]
Anagen			
Jan.	0.00	0.00	0.00
Feb.	0.00	7.70	5.60
Mar.	0.00	0.00	0.00
Apr.	15.40	5.55	13.00
May	27.30	25.00	20.08
June	0.00	5.27	3.44
July	0.00	28.60	44.45
Aug.	88.88	88.88	88.88
Sept.	100.00	75.00	83.00
Oct.	0.00	0.00	0.00
Nov.	0.00	20.00	10.00
Dec.	0.00	0.00	0.00
Catagen		Not detected in the sample	
Telogen			
Jan.	100.00	100.00	100.00
Feb.	100.00	92.30	94.40
Mar.	100.00	100.00	100.00
Apr.	84.60	94.45	87.00
May	72.70	75.00	79.92
June	100.00	94.73	96.56
July	100.00	71.40	55.55
Aug.	11.12	11.12	11.12
Sept.	0.00	25.00	17.00
Oct.	100.00	100.00	100.00
Nov.	100.00	80.00	90.00
Dec.	100.00	100.00	100.00

[a]Includes unsexed specimens.

was still apparent in some individuals in November. From December through April almost all specimens were in telogen.

From the specimens sampled, no latitudinal differences in the moult period were noted. Specimens from Alaska's Glacier Bay and from the Gulf Islands of British Columbia were in similar stages at the same time in spite of differences in season lengths and amounts of daily light. The failure of the sample to indicate sex and age differences in moult timing (with the exception that pups-of-the-year were all in telogen) was equally surprising. Scheffer and Slipp (1944) noted that Havinga had written of *P. v. vitulina* of Holland in 1933 that the yearlings moulted in July. The adults moulted in August and September. At Tugidak Island, Alaska, Mr Henry Hansen noted on May 25, 1965 that young harbour seals identified as "yearlings" were conspicuously shedding although the adults showed no outward signs of moult. Sex and age differences have been recorded for dates of moult in other phocids (Ling, 1965) and in otarids (Rand, 1956).

The length of time that *P. v. richardi* remains in each of the stages of the moult cycle has not been determined. Scheffer and Slipp (1944) noted from external evidence that moult in a captive specimen was completed in about 5 weeks. Montagna and Harrison (1957) found that plucked hair follicles required about 3 weeks for establishment of the bulb and a further 3 weeks for the new hair to reach the epidermal surface. Shedding occurs during anagen. Examin-

ation of "shedder" skins from which the old hairs can be shaken, brushed, or pulled reveals that the securely fastened hair of the new growth is about half the length or less, of the old hair. The mechanism of shedding is not understood. The thickened epidermal collar which surrounds the follicle below the orifice (Montagna and Harrison, 1957) loosens and the brush end of the club hair (Fig. 2) pulls loose with some of the adjacent cells attached (J. K. Ling, personal communication). At this time the developing hairs of the new pelage are firmly attached to the bulbs from which they are growing.

Catagen, the stage of regression of the hair bulb and dermal papilla(Montagna and Van Scott, 1958; Bullough and Laurence, 1958) is of very short duration in phocids (J. K. Ling, personal communication) and has not been identified in the specimens studied. The active hair bulb and papilla of anagen do not regress in *P. v. richardi* to the same extent as in species illustrated or described in the literature of hair development (Montagna and Van Scott, 1958; Bullough and Laurence, 1958; Rook, 1965) and tends to persist in reduced form throughout telogen (Fig. 2).

The adult moult is patterned, as in many other mammals (Scheffer, 1961; Crowcroft, 1957; Morejohn and Howard, 1956; Basset and Llewellyn, 1948) and differs in sequence (Fig. 3) from the intrauterine moult of the foetus (Fig. 4)

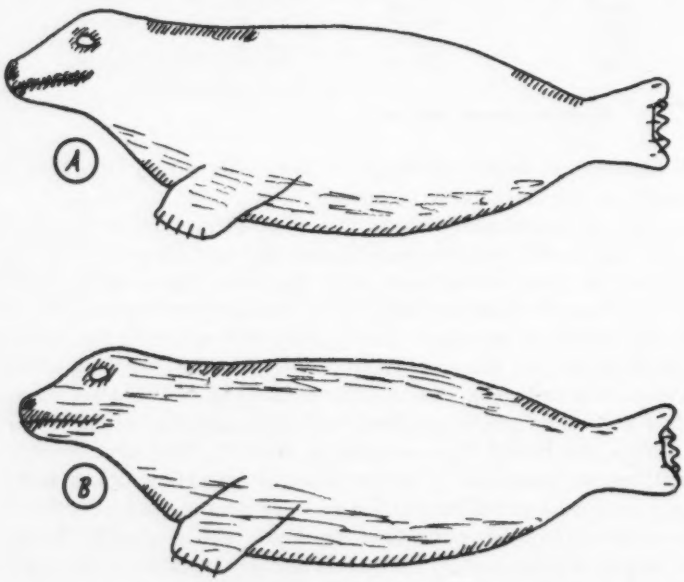

FIG. 3. Typical moult progression in the harbour seal of British Columbia. A, early moult. Diagonal shaded areas around eye, nose, mouth, and ventral and dorsal surfaces are shedding old hair and exposing short new hair. Lightly shaded region of flank is zone of conspicuously faded and worn hair. B, a later moult stage. New hair has replaced old hair in the diagonally shaded areas. Shedding is in progress in the lightly shaded areas and spreading laterally.

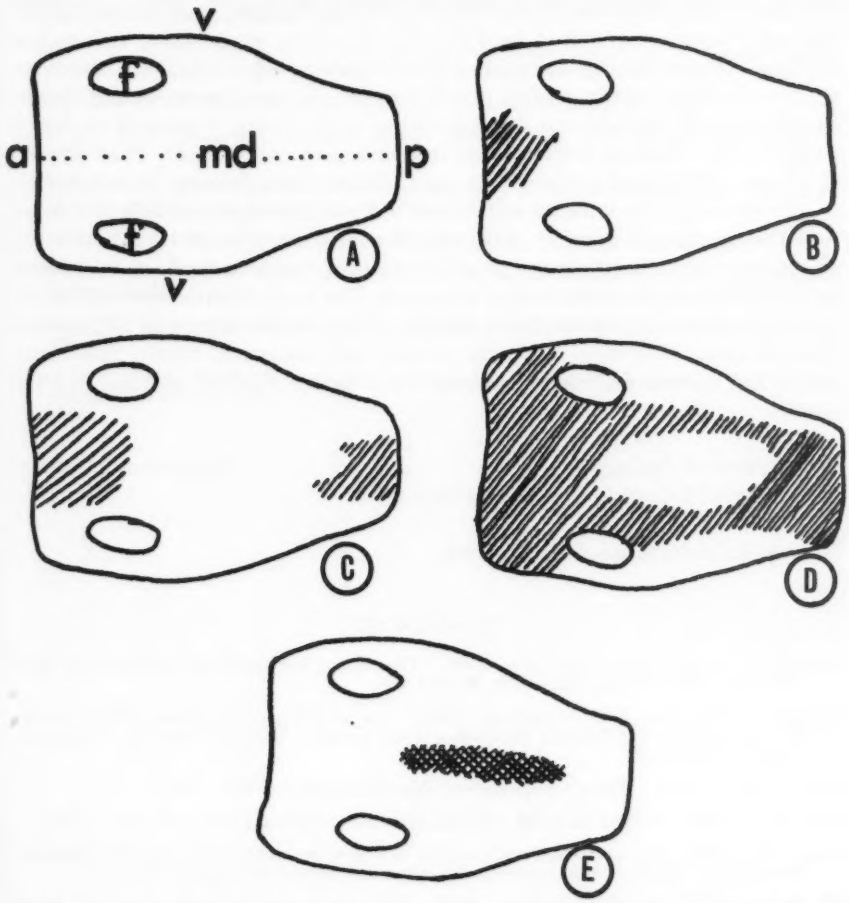

FIG. 4. The foetal moult pattern in *P. v. richardi*. A, outline drawing of typical seal pelt after removal from the carcass. Key: *a*, anterior; *f*, opening at anterior flipper site; *md*, middorsal line; *p*, posterior; *v*, ventral midline. B, the lined area plus the anterior flippers and the face is the first part of the foetal pelt to moult the lanugo hair. C, D, later stages of the foetal moult. Note that the ventral and middorsal areas retain lanugo hair until late in the moult. E, the cross-hatched portion of the lumbar middorsal region is the last area to shed the foetal hair.

(Stutz, 1966). My observations indicate that the general pattern of the moult agrees with the description of a captive seal by Scheffer and Slipp (1944). The first areas to replace the hair are around the body openings (anus, eyes, penial opening, ears). Moult of the body itself begins along the ventral midline and on the anterior (neck) and posterior (tail) dorsal midline areas and progresses as a wave of development and shedding until the moult is complete (Fig. 3). Much individual variation in moult pattern has been noted.

Some skins, classified by pelt buyers as "total shedders," moult haphazardly and may have various-sized bald patches in which regenerating hair is not apparent. These may reflect pathological conditions. In England a specimen of *P. v. vitulina*, captured when about 2 weeks old, was toothless and almost completely bald and did not develop hair or teeth during 3 years of captivity (King, 1964). Baldness has also been reported in the ringed seal, *Pusa hispida* (McLaren, 1958) and in the ribbon seal, *Histriophoca fasciata* (Roest, 1964).

In summary, the harbour seal possesses a complete mammalian hair system (less the erector pili muscle). The annual moult sequence of telogen (resting phase) to anagen (development phase) to catagen (regression phase) to telogen resembles the moult cycles of other mammals. The moult is patterned and occurs primarily during the summer and fall with its peak in August and September. There is much individual variation in date and pattern of moult. Specimens exhibiting varying degrees of baldness occur in the harbour seal and related species.

Department of Zoology
University of British Columbia, Vancouver, B.C.

SHERWOOD S. STUTZ[1]

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[1]Present address: Headquarters, Modoc National Forest, P.O. Box 1741, Alturas, California 96101.

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Serological Differences between two Populations of Oysters (*Crassostrea virginica*) from the Atlantic Coast of Canada

An epidemic disease of oysters, commonly known as Malpeque disease, has severely depleted oyster stocks in eastern Canada. One of the features of epidemics of this disease is that the survivors and their progeny are disease resistant. In contrast, oysters transplanted from disease-free areas contract the disease and die, with a recorded mortality of higher than 90% after 2 years' exposure (Needler and Logie, 1947; Logie et al., 1960; Drinnan and Medcof, 1961). Apparently, survival depends upon inherent resistance. Loosanoff, as quoted by Numachi (1962), indicated that physiological differences may exist within the same species of oysters. Differences between antigenic components of four races of Japanese oysters (*Crassostrea gigas*) have been shown by Numachi (1959, 1962), and Hillman (1964) has reported that distinctly different chromatographic patterns of free amino acids and small peptides existed in two different stocks of *Crassostrea virginica*. Our preliminary work (unpublished results) on the cross-precipitation of oyster sera from disease-susceptible (S) and disease-resistant (R) stocks with their respective rabbit antisera (SAB, RAB) showed that the ring test titre of the homologous antigen–antibody reaction of the resistant stock (R-RAB) was much higher than the titre of the heterologous reaction with the antiserum prepared from the antigen of the susceptible oyster stock (R-SAB). However, these was no significant difference between the two reactions with the antigen from the susceptible stock (S-SAB; S-RAB). The present report describes further studies of the differences in the antigenic components of the two populations of oysters using agar gel diffusion tests.

The oysters used for these experiments were 8 cm or larger in length and had been maintained in our aquarium (Stewart and Power, 1963) for several weeks at approximately 10 C. Two sets of pooled oyster sera were used as antigens. One set was drawn from 167 apparently healthy oysters of disease-susceptible stock from Bras d'Or Lake, Nova Scotia, and the other set was drawn from 202 healthy oysters of disease-resistant stock from Malpeque Bay, Prince Edward Island. The debris in the oyster sera was removed by centrifugation at 4 C, and the antigens were standardized in terms of their protein concentration (13.5 mg/ml) using the method of Greenberg (1929). Merthiolate[R],[1] 100 ppm, was used as a preservative. Antibodies against the oyster antigens were produced in rabbits by a standard immunization procedure which included an intravenous presensitizing inoculation and subsequent subcutaneous injections of antigens in Freund's incomplete adjuvant in a series of graded doses (Numachi, 1962). The antisera were harvested by

[1]Merthiolate[R], Eli Lilly and Co., Indianapolis, Indiana.

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the standard procedure described in the Manual of Microbiological Methods (Society of American Bacteriologists, 1957) after a satisfactory titre had been reached.

The balance of the reacting system is usually an important factor in the diffusion pattern development; consequently, the antigens were (*a*) concentrated by dislysis against carbowax (Miyamoto et al., 1957), or (*b*) diluted in a buffered saline (*p*H 7.0) before being reacted with the antisera. No improvement in resolving power was observed using either of these techniques. Thus, in the subsequent experiments described below, the antigens (13.5 mg/ml) and antisera were used in their natural state.

Noble agar (1.5%; Difco) without salt was used as the gel diffusion medium for testing the antisera against the original antigens. Merthiolate^{R} and methyl orange were added to the medium in final concentrations of 100 ppm and 30 ppm, respectively. The gel plates were prepared according to the methods described by Wilson and Pringle (1954). The antigen wells and antiserum wells were filled once. The precipitation lines were observed after the plates had been incubated at 20–25 C in a humid atmosphere for 5–10 days.

The results of the agar diffusion tests are shown in Fig. 1. When the antigens (R and S) were reacted with the antiserum to the disease-resistant stock antigen (RAB), the presence of two major precipitation lines was recorded for the homologous R-RAB reaction (Fig. 1A), whereas only one major precipitation line was noted for the heterologous S-RAB reaction. The formation of a regular arc of interference by the slow diffusion major lines of the two antigens indicated that a common antigenic component is shared by both oyster stocks. The corresponding fast diffusion major line, present in the homologous R-RAB reaction and absent in the heterologous S-RAB reaction, indicated the presence of an antigenic component in the disease-resistant oyster stock which is lacking in the disease-susceptible stock. When the antigens were reacted with the antiserum to the disease-susceptible stock antigen (SAB), only the slow diffusion major precipitation line, plus a number of minor lines, was seen in both the homologous S-SAB and heterologous R-SAB reactions, indicating that there are no components in the S antigen which are not also present in the R antigen (Fig. 1B)

To test the consistency of agar gel diffusion patterns of the two populations of oysters, the antisera were reacted with freshly prepared antigens from the two oyster stocks. A total of three trials have been completed and the results are summarized in Table I. This particular fast diffusion major precipitation line was consistently found in the homologous R-RAB antigen–antibody reaction, and was non-existent or present at an insignificant level in the heterologous S-RAB reaction. This line was never found in any reaction using the disease-susceptible antiserum (SAB). Our limited data would suggest that an antigenic difference exists between the two populations of oysters. Many factors such as diet, temperature, salinity, and maturity may have effects on serum protein composition (Hillman, 1964). Consequently care should be taken in interpreting the results of immunological studies.

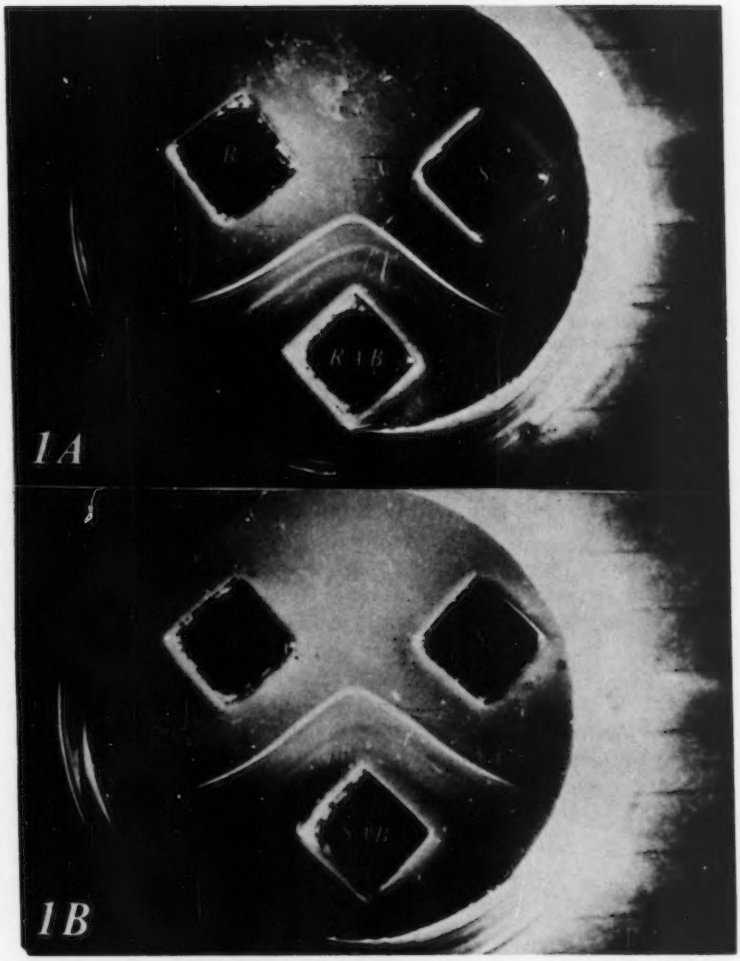

FIG. 1. Agar gel diffusion showing the reactions (A and B) between antigens from the disease-resistant and the disease-susceptible oyster stocks and their homologous and heterologous antisera: R, antigen from the disease resistant oyster stock; S, antigen from the disease susceptible oyster stock; RAB, antiserum to the antigen from the resistant stock; SAB, antiserum to the antigen from the susceptible stock.

TABLE I. Agar gel precipitin tests with oyster antigens from the two different stocks.

		Antisera	
	Antigens[a]	SAB	RAB
Trial 1	S[b]	–[c]	–
	R	–	+
Trial 2	S	–	–
	R	–	+
Trial 3	S	–	±
	R	–	+

[a]Each antigen was freshly prepared from pooled sera from six oysters of the same stock.
[b]S, antigen from the susceptible stock; R, antigen from the resistant stock; SAB, rabbit antiserum to S; RAB, rabbit antiserum to R.
[c]+ indicates the presence of the fast diffusion major precipitation line; – indicates the absence of this line; ± indicates that this line was barely detectable.

However, in view of the consistency of the immunological patterns of the two stocks and the variability in disease resistance, the results would suggest that these stocks are genetically different.

Further immunological studies of these two oyster populations by absorption tests and immunoelectrophoretic techniques are in progress to determine more precisely the nature of the antigenic components and their relation, if any, to disease resistance.

We thank Mr R. E. Drinnan, Fisheries Research Board of Canada, Ellerslie, P.E.I., for bringing the disease problem to our attention, supplying the information on the epidemiological background, demonstrating the histological and blood collection techniques, and supplying the oysters for these experiments.

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

M. F. LI
CAROL FLEMMING
JAMES E. STEWART

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The Urinary and Genital Systems of Trout

A complete and accurate description of the reproductive and excretory systems of the Salmonidae was given by Weber (1887) in the manner of the great German anatomists. Unfortunately, his somewhat lengthy paper has been largely overlooked and modern literature frequently contains erroneous statements relating to the anatomy of the reproductive and urinary systems.

In two papers concerned with peritoneal membranes, Kendall (1920a, b) described and illustrated functional oviducts in the salmonids. According to Kendall, ova are not released into the abdominal cavity, but are conveyed to the exterior by oviducts which extend from the gonads to the genital opening. "The prolongation backward of the mesovariums and ovarian investments form the oviducts, which in the Salmonidae and Coregonidae are trough-like, open above, the inner wall consisting of the mesovarium and the free outer wall supported by the abdominal wall. Near the outlet, the two troughs unite into one above the intestine at the point of termination of the dorsal mesentery. At a short distance from the genital orifice each outer wall of the common channel is deflected and is attached to the respective wall of the abdomen." (1920a, p. 200.) Kendall's work is cited in Berg's (1947) "Classification of Fishes" and is undoubtably the source of similar incorrect references to oviducts which appear from time to time in current publications.

The urogenital sinus is a term which, according to common usage, describes an internal cavity into which the urinary and genital products are discharged before being released to the exterior. Among others, Parker and Haswell (1940) state that a urogenital sinus is present in *Salmo fario* (= *S. trutta*).

Parker (1943) drew attention to the anatomical errors mentioned above in connection with *S. fario;* however, his photographic illustrations are lacking in detail. It is hoped that this brief communication will provide a simple and clear account of the two systems. The following description is based on dissections of *Salvelinus fontinalis*, *S. namaycush*, *Salmo trutta*, and *S. gairdneri*.

In female trout, each ovary is supported by a double layer of peritoneum, the mesovarium. The mesovarium originates from the ventro-lateral peritoneum covering the swimbladder and extends to the ovary where it separates into the peritoneal membrane enveloping the gonad. The two mesovaria extend caudally in the abdomen from the posterior ends of the ovaries as thin membranous strands. These are the peritoneal or mesovarial folds and are the structures which Kendall has termed oviducts. They are continuous laterally with the parietal peritoneum lying against the swimbladder. Near the posterior limit of the abdominal cavity, the peritoneal folds turn medially and appear to unite with the dorsal mesentery which supports the intestinal tract.

The peritoneal folds are not functional gonaducts. When ripe, the ova are discharged from the gonads directly into the abdominal cavity and pass

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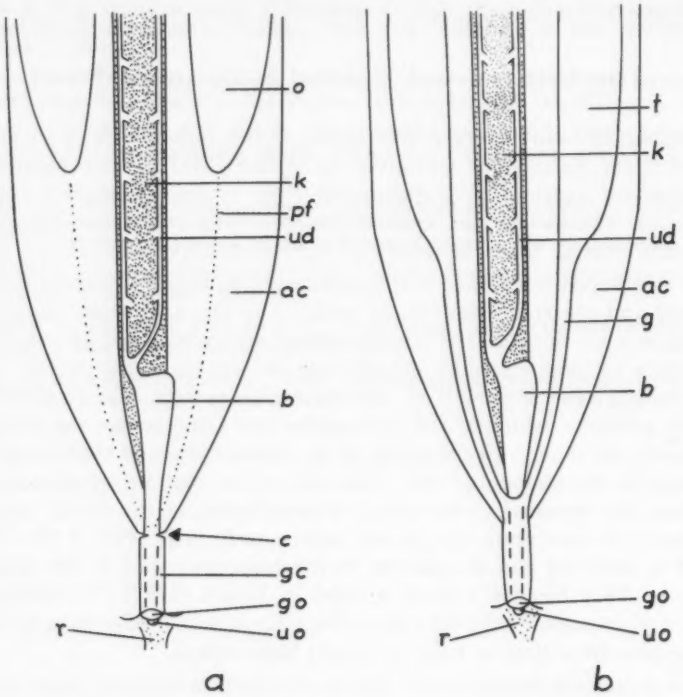

FIG. 1. The urinary and genital systems of (a) female and (b) male trout: *ac*, abdominal cavity; *b*, bladder; *c*, genital constriction; *g*, gonaduct; *gc*, genital cavity; *go*, genital orifice; *k*, kidneys; *o*, ovary; *pf*, peritoneal fold; *r*, recess; *t*, testis; *ud*, urinary duct; *uo*, urinary orifice.

through a constriction in the posterior abdominal wall into a cavity that opens to the exterior through an orifice on the urogenital papilla. (Occasionally, the coelomic constriction is double rather than single.)

The urinary ducts unite to form a single channel near the posterior end of the kidneys. This duct opens into a bladder which follows the dorsal wall of the abdominal cavity. It extends back above the coelomic constriction and genital cavity to terminate in a small orifice immediately posterior to the genital opening on the papilla.

In male trout, milt is discharged into thick-walled gonaducts which extend posteriorly from each testis. Like the peritoneal folds in females, the gonaducts are posterior extensions of the gonadal mesenteries or mesorchia and are continous laterally with the parietal peritoneum covering the lower surface of the swimbladder. The two gonaducts join before leaving the abdominal cavity to form a sinus that passes back to open by an orifice on the urogenital papilla. As in females, the urinary opening is directly behind the genital pore.

In both sexes, the urogenital papilla lies in an external cup-shaped recess just behind the rectal opening. This depression should not be called a urogenital sinus since it does not serve as a passageway for the exit of urinary and genital products from the interior to the exterior of the fish.

Department of Biology
University of Calgary, Calgary, Alberta

NANCY E. HENDERSON

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Pelage Patterns and Population Distributions in the Pacific Harbour Seal (*Phoca vitulina richardi*)

Harbour seals (*Phoca vitulina*) are described and illustrated widely in the literature. The species exhibits wide variation in colour and spot patterns and both very light and very dark specimens are found in the same or closely adjoining regions (Scheffer and Slipp, 1944). Colour has been used to separate subspecies. *Phoca vitulina geronimensis* was distinguished from *P. v. richardi* by its allegedly darker colour (Scheffer and Slipp, 1944; Schwarz, 1942). The dark colour described as typical of the shore-pupping form of the Kurile Islands was used by Belkin (1964) as a taxonomic device for separating *Phoca insularis* from the ice-pupping form, *Phoca vitulina largha*.

Except for the whitecoat pup of *P. v. largha* (Wilke, 1954; King, 1964) and occasional white pups in other subspecies (Stutz, 1966), the coats of all postnatal harbour seals fall into the three categories described below. Figures 1 and 2 (supplied by Dr R. J. Harrison of the London Hospital Medical College, England) indicate that the pattern persists through successive moults. In these photographs, taken in 1960 and 1962, the distinctive pattern of spots on the "chest" anterior to the left pectoral flipper are clearly visible and clearly identical.

Examination and photography of 300 harbour seal pelts from various Pacific locations indicated three categories, "black," "common," and "muddy," in which all skins could be placed by pattern and colour. Descriptions are as follow: *black* — black or very dark brown on both dorsum and venter. Marks white, distinct (Fig. 3); *common* — bluish-gray dorsum, varying from almost white to very dark. Flanks and venter paler than dorsum, usually white. Spots on flanks and venter may be bluish-gray or brown. Spots on dorsum may be black, brown, or white. Marks are distinct (Fig. 4); *muddy* — mottled shades of indistinct pattern and vague markings. Pale shades of mingled gray–beige–white with dorsum darker in neutral shade, frequently without marks on venter; or dark bluish-brown shade of dorsum with lighter venter and indistinct mottled markings (Fig. 5). The distribution of these categories varied between regions of the coast. To the three natural colour types should be added a fourth or artifact colour type. The so-called "Alaska golden sealskins" are never found on living seals, wet or dry. They result from improperly processed raw skins where the hair has been smeared with blubber grease and allowed to dry (Dunbar, 1949). The yellow colour can be prevented by carefully removing all blubber from the hide and washing the pelt in detergents to remove fat that has been rubbed onto the hair. "Oxidized" (yellow) skins bring lowered market prices since European processors dye them to conceal the faulty colour.

Dunbar (1949) and Mansfield (1963) writing of *P. v. concolor* of eastern Canada commented that harbour seals were much darker in the northern parts of their range than their conspecifics farther south. This trend does not appear

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among Pacific harbour seals. The harbour seal of southern California and northwestern Mexico was formerly classified as *P. v. geronimensis* (Miller and Kellogg, 1955) on the basis of its dark colour. I have examined both very dark and very light skins from Washington State, British Columbia, and several Alaskan locations.

Table I indicates the colour patterns and geographic origins of 247 seal pelts. The pattern of distribution of the black and common skins was tested for significance with an "F test" analysis of variance (Li, 1964). The results indicated significant differences in the distribution of black (F equals 4.06) and common (F equals 3.66) skins. A test of the distribution of muddy pelts revealed no differences.

Duncan's multiple range test (Li, 1964) indicated statistically significant differences between the means of the black components of the Queen Charlotte Islands, Glacier Bay, and the Strait of Georgia samples. The means of common patterned skins differed significantly between the Queen Charlotte Islands and Strait of Georgia samples, but not between those areas and the Glacier Bay sample.

It is probable that the colour patterns in *P. vitulina* had some adaptive significance in the past. There is no reason to believe that, with the exception of *P. v. largha's* whitecoat pup, which is exposed on ice (Wilke, 1954; King, 1964), there is now any adaptive significance in the distribution of pelage patterns in this species. The lack of adaptive significance in the distribution of pelage patterns is a clue to the separation of *P. v. richardi* into separate breeding stocks.

The distribution of pelage types by area (Table I) demonstrates that Glacier Bay and vicinity in Alaska, the Queen Charlotte Islands, and the Strait of Georgia have populations which differ genetically. Each of these areas can be considered a discrete entity. The narrow continental shelf which is bordered by a huge abyssal plain (Ũda, 1963) and the ecological characteristics of the seal are responsible for the separation of breeding populations.

Scheffer and Sperry (1931) reported that harbour seals fed primarily on fishes and invertebrates of shoal water bays and surface waters offshore. The seals commonly fed in the tidal areas about stream mouths (Scheffer and Sperry, 1931). Scheffer and Slipp (1944) stated that the harbour seal was seldom reported beyond 15 miles from shore. Spalding (1964) reported that the harbour seal was rarely seen more than 5 miles from shore although some were observed with other species at the Portlock Bank in the Gulf of Alaska between 30 and 40 miles from shore in June 1962. The evidence supports a view that the harbour seal is a species of inshore and primarily shallow waters.

The tendency to use shallow waters, close to shore, is a probable reason for *P. vitulina's* failure to cross the 170-mile gap that separates Guadalupe Island from the Baja California mainland. Scheffer (1958) noted the presence of other pinnipeds and the absence of the harbour seal from Guadalupe. The narrow linear habitat has divided the seal population into separate gene pools. Glacier

FIG. 1. Three-year-old female common seal, *Phoca vitulina vitulina*, photographed November 1960. Compare the spot pattern visible anterior to the left front flipper with Fig. 2. FIG. 2. Five-year-old female *P. v. vitulina* photographed August 1962. This is the same seal as illustrated in Fig. 1. Note the unchanged spot pattern anterior to the left front flipper. (Photographs by courtesy of Professor R. J. Harrison, London Hospital Medical College.)

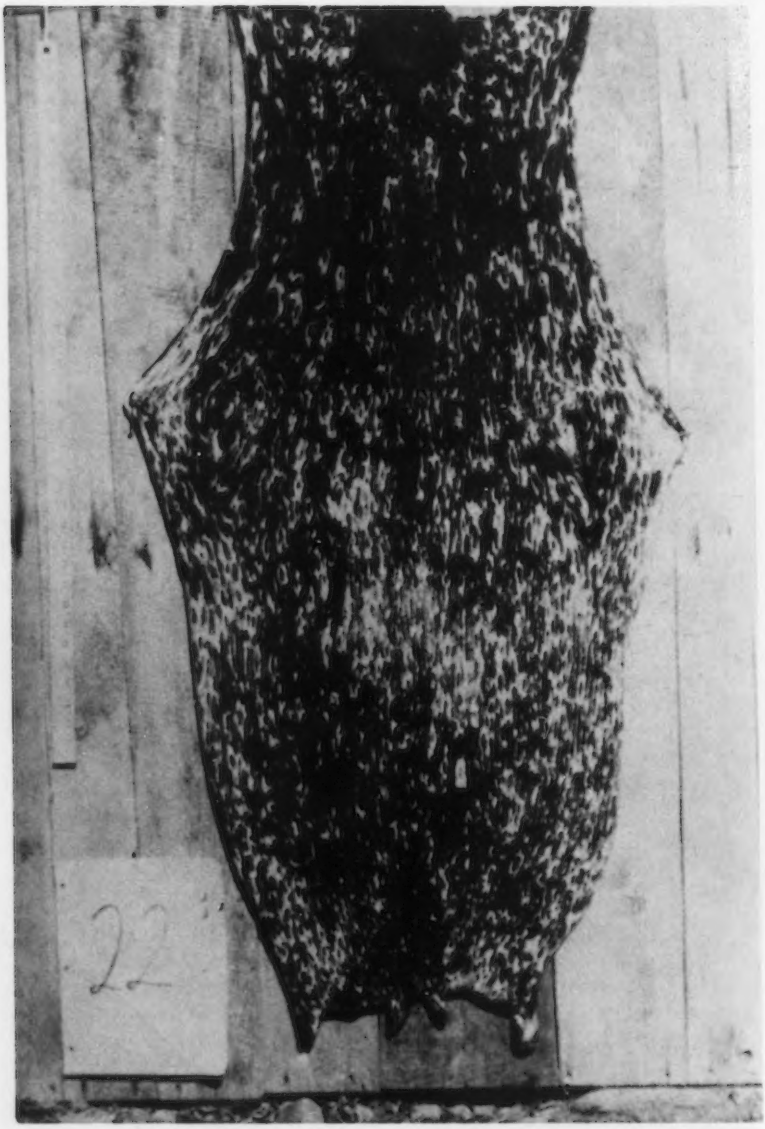

FIG. 3. Black pelt, male, collected at Sullivan Bay, B.C., early May 1964.

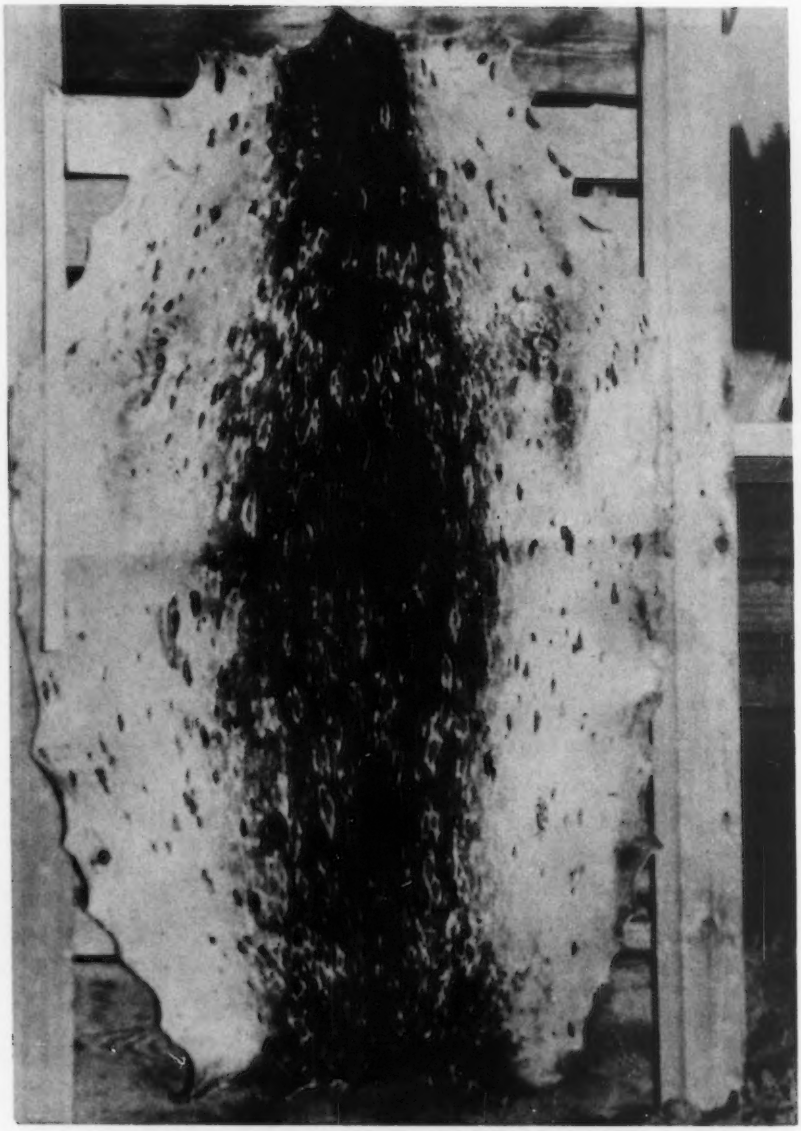

FIG. 4. Common pelt, male, collected near Nome, Alaska, October 1964.

FIG. 5. Muddy pelt, male, collected at Rivers Inlet, B.C., early August 1964.

Stutz — J. Fish. Res. Bd. Canada

TABLE I. Distribution of pelage types by area.

Type		Queen Charlotte Islands, B.C.	Glacier Bay, Alaska	Strait of Georgia, B.C.	Knight Inlet, B.C.	Bering Sea	Skeena–Prince Rupert, B.C.	Juneau, Alaska	Totals
Black	No.	50	6	17	7	7	4	5	96
	%	55.56	18.75	23.61	50.00	41.18	33.33	50.00	
Common	No.	14	12	33	2	8	4	3	76
	%	15.55	32.50	45.83	14.29	47.06	33.33	30.00	
Muddy	No.	26	14	22	5	2	4	2	75
	%	28.29	43.75	30.56	35.71	11.76	33.33	20.00	
Total numbers		90	32	72	14	17	12	10	247

Bay is located at the northern end of the "Inside Passage" and opens southward into the northern end of the Alexander Archipelago. The Queen Charlotte Islands lie south of the Alexander Archipelago and west of the northern British Columbia coast and are nowhere closer than about 30 miles to either the Alaskan islands or the outer islands of the B.C. mainland. The Strait of Georgia comprises an enclosed and rather lake-like waterway about 120 nautical miles long, an average of 18 nautical miles wide, with a mean depth of 85 fath (Waldichuk, 1957). For analysis of seal populations I have included Puget Sound and the waterways north to Sointula in the "Strait of Georgia."

Between Olympia, Washington and Sointula, B.C., there are no natural barriers or natural defiles restricting or constricting the free flow of genes in the *P. vitulina* population. There is no true migration in harbour seals, but the wide overlap in home ranges coupled with a preference for certain types of habitat at various seasons (Spalding, 1964) would keep the seal population of the Strait of Georgia area continuously mixed and prevent the isolation of breeding groups from the remainder of the regional population.

The Queen Charlotte Islands form a geographical and ecological unit which probably does have some influence on the population of the nearby mainland shore. Transfer of individuals between the coastal island fringe and the Queen Charlotte Islands is probably irregular and in small numbers since the average distance would be about 50 miles.

The transfer of genetic material between the north and south along the coast of the northern B.C. mainland and the west coast of Vancouver Island and Washington State must be limited to a relative trickle along the narrow edge of the continental shelf. Areas such as Glacier Bay, the Queen Charlotte Islands, and the Strait of Georgia would therefore be relatively isolated centers of large breeding populations separated by stretches of narrow zones of harbour seal habitat. These narrow zones would act as natural defiles and reduce the incidence of gene transfers across their areas.

H. D. Fisher (personal communication) has described the harbour seal of the western Atlantic as almost uniform in colour pattern as contrasted with the variation noted along the Pacific coast. On the North American Atlantic coast the existence of plentiful areas suitable for harbour seal havens plus a wide, shallow continental shelf and extensive offshore banks permits unrestricted mixing of stocks from the entire coast and prevents the development of populations of visibly different genetic makeup.

Department of Zoology
University of British Columbia, Vancouver, B.C.

SHERWOOD S. STUTZ

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Dimethyl-β-Propiothetin Dimethyl Sulphide in Labrador Cod

The occurrence of the "blackberry" problem, a pronounced and offensive odour of dimethyl sulphide (DMS) in Labrador cod, was first brought to our attention in 1963 (Sipos and Ackman, 1964), but subsequently failed to appear on a large scale in the fishery until 1966.

In feeding experiments with laboratory-held cod, Ackman et al. (1966) established that an intake of approximately 10 mg/day of dimethyl-β-propiothetin (DMPT), the known precursor of DMS, was necessary to deposit DMPT in muscle tissue of market-size cod under the particular holding conditions. Cooking experiments indicated that residual DMPT could affect the taste of cooked cod under certain circumstances. It was, therefore, considered desirable to determine DMPT levels in cod taken while feeding on *Limacina helicina* or related pteropods, the zooplankton particularly associated with the "blackberry" problem (Sipos and Ackman, 1964; Ackman et al., 1966; Lippa, 1965; Motohiro, 1962).

Limacina helicina, regarded as an Artic species (e.g. Dunbar, 1942), is distributed along most of the eastern Canadian coastline (Kerswill, 1940) in water of Arctic origin. Along the Labrador coast it is most abundant in late summer. Relative abundance varies from year to year, being scace in 1964 and 1965 but plentiful along the whole coast in 1966. Whether this is a distributional anomaly caused by variations in prevailing winds or currents or a real change in abundance is not known.

When plentiful they are frequently found in stomachs of cod and of capelin. The resultant offensive odour and taste is not retained in salt-cured cod but could affect the frozen product.

Samples and Analyses — Cod were taken from cod traps in the course of routine investigations of this fishery. Owing to the limited freezing space available only parts of larger single specimens could be frozen for this study. These fish were selected at random from those with a marked "blackberry" odour. The stomach contents of the catches from which specimens 1, 2, and 3 were taken consisted entirely of pteropods. A few fish in the catch from which specimen 4 was taken also contained ctenophores, and many in the catch from which specimen 5 was taken also contained capelin. Statements on the date and location of catch, and on particulars of individual fish, are given in Table I.

The fish were eviscerated, and the body or fillets, liver, and remaining viscera placed in separate plastic bags. The samples were then frozen and shipped in dry ice and held at −20 C for a few days until analysed. Stomachs were separated from other viscera for analysis.

Analyses were carried out on filtrates from blends prepared at room temperature with chilled distilled water using procedures already described (Ackman et al., 1966). The visceral materials contained large amounts of DMS. To obtain

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TABLE I. Background data for specimens included in analysis.

Fish No.	Date	Area	Depth (*fath*)	Sex	Length (*cm*)	Whole fresh weight (*g*)	Weight liver (*g*)	Weight stomach (*g*)
1	July 2/66	St. Anthony	10	?	30	263	12	28
2	July 21/66	Pack's Hr.	8	M	40	600[a]	18	54
3	July 21/66	Pack's Hr.	8	M	40	600[a]	41	29
4	Aug. 2/66	Black Is. (Nain)	10	F	56	1550[a]	64	137
5	July 9/66	Pack's Hr.	7–8	M	37	480[a]	14	15

[a]From empirical length–weight curve for Labrador area.

the amount of DMS liberated by alkali from DMPT in the presence of this DMS, the sample was placed in the reactor vial and the vial attached to the reactor (Ackman and Dale, 1965), the vial then being pressurized with helium and stirred for 2 min, followed by injection of head space gas into the gas chromatograph. Immediately stirring was recommenced and after 2 min head space gas was again injected. This procedure, repeated three or four times, gave a series of DMS peaks of descending magnitude (Fig. 1). Finally, following the same time sequence, normal addition of alkali solution liberated additional DMS from DMPT. The heights of the preliminary DMS peaks were then adjusted for baseline drift (occurring in this apparatus while the recorder chart and pen drives were switched off until the moment of gas injection). A line was drawn through the adjusted peak tops of the DMS peaks intercepting the DMS peak after alkali injection. The additional DMS contribution from DMPT observed above the intercept was then used for determination of the latter.

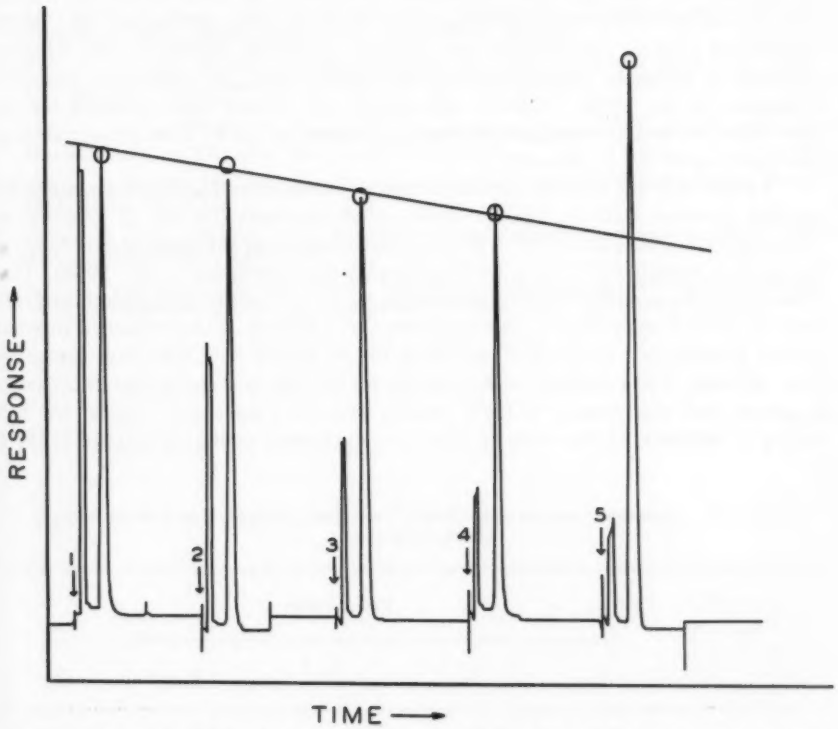

FIG. 1. Sequence of injections of preexisting head space gas containing dimethyl sulphide (DMS) (points 1–4 followed by injection of head space gas including additional DMS generated from dimethyl-β-propiothetin (DMPT) by alkali (point 5). Peak heights corrected for baseline drifts are marked as O.

This procedure does not allow specifically for diminution in head space volume after addition of the alkali, or for temperature rise due to heat of mixing of concentrated alkali solution in the filtrate. Tests indicated that these variables, which possibly cancel each other, had little effect on the accuracy of DMPT determination. The errors introduced were negligible in comparison with possible losses of DMPT through thermal degradation in removal of the DMS by other means (Ackman and Dale, 1965; Ackman et al., 1966; Motohiro, 1962). DMPT contents of tissues (Table II) are given as free DMPT and are accurate only to $\pm 20\%$.

Only traces of DMS were observed in the cod flesh samples in comparison with the stomachs and livers from the same fish. These relative levels are of only qualitative significance, since storage and operations of blending and aspirator-vacuum filtration would lead to considerable losses. The livers might be expected to retain DMS longer than flesh by virtue of the greater solubility of DMS in oils, and free DMS levels in liver blends ranged from one-quarter to one-half of those of stomach blends. DMS may be detected by the human nose at remarkably low concentrations, with a lingering aftereffect, and in the vicinity of gutting and filleting operations it would be difficult to tell whether fillets were seriously contaminated with DMS or not. As previously observed (Ackman et al., 1966), DMS is apparently not formed from DMPT by the live fish; the enzyme responsible for breakdown of DMPT is presumably in the algae eaten by *L. helicina*.

Levels of DMPT in the stomach contents of these cod are of the same order as that reported (0.01 mg/g) for the stomach contents (largely *L. helicina*) of chum salmon (Motohiro, 1962), but markedly less than the level (0.2–0.5 mg/g) found in a sample of *L. helicina* from Baffin Bay (Ackman et al., 1966). This level would be modified by the proportion of *L. helicina* to other food, and the level of DMPT may vary in this organism. In addition, it is not known to what extent degradation of DMPT continues in the stomach of fish after ingestion of *L. helicina*. The similarity of the results for salmon and cod stomach contents suggests that the present DMPT results may be considered typical for fish eating *L. helicina*. If this view is valid, the maximum probable level of DMPT

TABLE II. Dimethyl-β-propiothetin (DMPT) contents of tissues (free DMPT in mg/g tissue) from cod.

	Fish number				
	1	2	3	4	5
Muscle	0.004	0.007	0.005	0.029	0.009
Liver	0.012	0.002	0.006	0.026	0.001
Stomach	0.048	0.009	0.016	0.030	0.005

in cod flesh as determined here would be only a tenth of that in fillets used in cooking experiments where deleterious effects on taste were observed. It is, therefore, unlikely that thetin deposits will be a serious problem in flesh from "blackberry" cod.

Fisheries Research Board of Canada
Halifax, Laboratory, Halifax, N.S.

R. G. ACKMAN
J. HINGLEY

Biological Station, St. John's, Nfld.

A. W. MAY

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Introduction of *Mysis relicta* Lovén into Kalamalka and Pinaus Lakes, British Columbia

In 1949 and 1950 *Mysis relicata* Lovén was introduced into Kootenay Lake (Larkin, 1951) to provide a supplementary food supply for rainbow trout (*Salmo gairdneri*). They remained undetected until reported by anglers in 1961, and in 1963 a successful introduction was confirmed (Sparrow and Larkin, 1964).

Mysis is an important item in diet of juvenile lake trout (*Salvelinus namaycush*) (Rawson, 1961), and there is some evidence to suggest that they may have contributed materially to an apparent increased growth rate of kokanee (*Oncorhynchus nerka*) in Kootenay Lake. Mature kokanee collected in 1951 did not exceed 250 g (Vernon, 1957), whereas one specimen captured at an egg-collecting station in 1965 weighed 3.6 kg and kokanee up to 3 kg were taken by anglers in 1966. Nevertheless, significant changes in mean growth rate of the population remain to be verified as well as the contribution of *Mysis* to kokanee diet.

British Columbia has innumerable oligotrophic lakes and many support slow-growing populations of rainbow trout or rainbow trout and kokanee. Purpose of the transfer of *Mysis* to Pinaus and Kalamalka lakes is to study subsequent effects on growth rate of these sportfish in the event the introductions prove successful. This note describes how the transfer was carried out.

Lake Characteristics — In September and October 1965, *Mysis* was transferred from Kootenay to Kalamalka and Pinaus lakes in south-central British Columbia. Kalamalka contains a large variety of fish species including rainbow trout, kokanee, and mountain whitefish (*Coregonus williamsoni*). Pinaus, however, contains only rainbow trout, having been chemically treated with toxaphene in 1958 (Stringer and McMynn, 1960). Both are oligotrophic, and Table I gives some of the physical and chemical characteristics.

TABLE I. Some physical and chemical features of Kalamalka and Pinaus lakes.

Lake	Mean depth (*m*)	Maximum depth (*m*)	TDS (*ppm*)	Area (*ha*)	Secchi disc (*m*)
Kalamalka	58.5	141.7	272	2569	9.8
Pinaus	22.3	53.6	140	168.8	7.0

Collection and Transportation — *Mysis* have been collected for transport using bottom trawls (Linn and Frantz, 1965; Furst, 1965). Bottom trawling operations are subject to considerable hazards such as snagging of gear or injury of mysids by mud compaction in the trawl. Since *Mysis* in Kootenay Lake undergo regular diurnal migration (E. R. Zyblut, personal communication)

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these problems are obviated if they can be collected at or near the surface. Thus, collections were made at night with a conical-shaped net (Fig. 1) similar to that described by Johnson (1956) but having a web of six knotless meshes per centimeter tied off 1 m from the cod end to reduce compaction. Horizontal tows were made, usually of 5-min duration with a few tows varying from 3 to 10 min. As many as 6000 *Mysis* were collected in one 5-min tow.

Collections for release in Kalamalka Lake, made in September during stormy weather with heavy cloud cover, indicated that *Mysis* were concentrated in the upper 5 m, and this greatly facilitated their capture. The net was hauled manually from the lake and inverted into plastic containers (Fig. 1) holding 50 to 60 liters of water. Towing speed was estimated at 3 knots.

Collections made the first night were held in the plastic containers without aeration and stored in a temperature control room at 9 C. Density of *Mysis* approximated 300/liter. After 10 hr, oxygen levels had dropped from 8.9 mg/liter to 0.96 mg/liter, and virtually all *Mysis* were dead. Subsequently, *Mysis* were transferred directly from the boat to a hatchery truck with a 189,600-liter insulated tank equipped with an aerating system (Fig. 1). A regulated flow of oxygen was bubbled into the water through a series of perforated plastic lines fixed to the bottom of the tank. Each net haul was inspected and it was evident that many mysids were injured, although extent of loss could not be judged for 3–4 hr, at which time dead specimens appeared white. Nevertheless, it was estimated after collection that about one-half of the *Mysis* loaded into the tank-truck were dead.

Kootenay Lake water (15.6 C) was used to fill the tank and unchlorinated ice was added which reduced the temperature to 8.3 C after about 2 hr. *Mysis* were in the tank for 44 hr, including 8 hr in transit, and water temperature varied from 8.3 C to 10.6 C. Oxygen concentration rose from 8.9 mg/liter to 13.2 mg/liter after 5 hr aeration, and this level was maintained until the *Mysis* were released.

At Kalamalka Lake, water was dipped from the tank and poured through seives. Sample counts were made to estimate total numbers and to determine the ratio of alive to dead. *Mysis* were acclimated for 1 hr in a 50 : 50 mixture of Kootenay and Kalamalka surface water. Approximately 46% were dead, indicating that loss through transportation was minimal. Surface temperature of Kalamalka was 16.7 C. An estimated 145,000 live organisms were released at the surface at midday in 83.8 m of water and they sounded immediately.

Collections for release in Pinaus Lake were made in October. In contrast to September conditions, the sky was clear with a full moon. Consequently, the main concentration of *Mysis* in Kootonay Lake ranged from 10 to 14 m and it was necessary to add weight to the net to reach required depth. The added weight increased the drag and reduced the towing speed to between 1 and 2 knots.

Since transport in this case was by aircraft (Cessna 195), *Mysis* were held in aluminum containers (generally used to transport small trout by aircraft) fitted with perforated plastic lines for aeration with oxygen (Fig. 1).

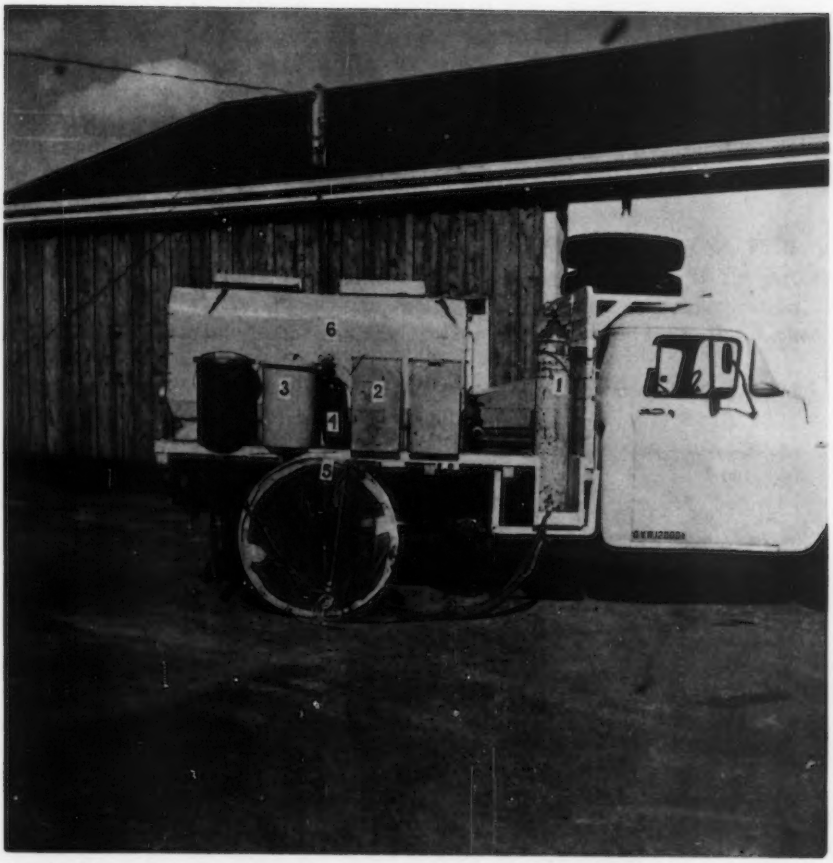

FIG. 1. Gear used in collection and transport of *Mysis*: 1, oxygen cylinder connected to bottom of tank via plastic hose; 2, aluminum container used in air transport; 3, plastic container carried in boat; 4, oxygen cylinder carried in boat and connected to containers; 5, tow net; 6, 189,600-liter tank.

Stringer—J. Fish. Res. Bd. Canada

Before take off, water in the eight cans was decanted to reduce weight, leaving some 30 liters per can. Concentration of *Mysis* during transit was as high as 500/liter. Surface temperature of Kootenay Lake was 14.4 C and temperature dropped during the night to 12.8 C in cans. Surface temperature of Pinaus Lake was 13.3 C. Oxygen concentration in the containers remained relatively constant at 11.6 mg/liter. Flying time was 40 min.

At Pinaus, two cans were arbitrarily selected and total counts made. In all, 20,102 were counted with a mortality of 10.5%. The reduced netting loss can probably be attributed to a slower towing speed (Furst, 1965). An estimated 80,400 *Mysis* were released at the surface of Pinaus Lake over a water depth of 53 m allowing 1 hr for acclimation in a 50 : 50 mixture of Kootenay and Pinaus water.

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Department of Recreation and Conservation G. E. STRINGER
Fish and Wildlife Branch, Kelowna, B.C.

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Fatty Acid Composition of the Decapod Shrimp, *Pandalus borealis*, in Relation to that of the Euphausid, *Meganyctiphanes norvegica*

Recently it was observed that the fatty acids of the zooplanktonic crustacean *Meganyctiphanes norvegica* (M. Sars) from Nova Scotia waters had a composition characterized by high proportions of monounsaturated fatty acids, specifically 20:1[1] and 22:1, and thus completely different from hitherto reported zooplankton fatty acid analyses (Ackman and Eaton, 1966). When samples of the commercially important (Squires, 1961; Butler, 1964) shrimp *Pandalus borealis* Krøyer were obtained in the course of exploratory trawling from approximately the same area (off the south coast of Nova Scotia), a comparison of the fatty acid compositions seemed desirable since almost all data for specific zooplanktonic crustaceans came from the North Pacific or Antarctic waters.

The shrimp were taken in late August 1965, and were held in ice for several days before landing. Since hydrolysis of specific lipids might have occurred in this period, a comparative analysis of fatty acids of the different types of lipid was not attempted. Berried females constituted one-third of the material obtained and the shrimp were sorted to obtain a sample free of berried females (A) and one solely of berried females (B). The average length was about 50 mm but it is not known what proportion of sample A was females (*cf.* Squires, 1961). Extraction by the method of Bligh and Dyer (1959), saponification of the lipid, and recovery of the nonsaponifiable material and fatty acids resulted in the data in Table I. Gas–liquid chromatography carried out as described elsewhere (Ackman and Eaton, 1966) gave the compositions for samples A and B listed in Table II.

TABLE I. Recovery and properties of lipids from extraction of *P. borealis* and *M. norvegica*.

	P. borealis		
	Sample A	Sample B	*M. norvegica*
Weight % lipid	2.43	2.21	2.19
Weight % non-saponifiable recovered from lipid	6.6	6.2	4.9
Weight % fatty acids recovered from lipid	75	76	94
Calc. iodine value of fatty acid esters	165	176	138

[1]Shorthand notation for chain length : number of double bonds. The addition of "ω" gives the position of the ultimate double bond from the terminal methyl group.

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TABLE II. Comparison of the composition of total fatty acids of *P. borealis* Krøyer and of *M. norvegica* (M. Sars), in weight per cent, by type of acid.

Fatty acid	*P. borealis* Sample A	*P. borealis* Sample B	*M. norvegica*
Saturated			
12:0	0.3	0.3	0.2
14:0	2.9	2.8	4.8
15:0	0.5	0.6	0.6
16:0	14.9	14.3	15.2
17:0	0.3	0.5	0.7
18:0	2.6	1.8	1.3
19:0	NSA[a]	NSA	Trace
20:0	Trace[a]	Trace	Trace
Σ	21.5	20.3	22.7
Monounsaturated[b]			
14:1	0.5	0.3	0.6
15:1	0.4	0.2	0.2
16:1	14.1	14.0	11.8
17:1	0.4	0.9	0.2
18:1	20.2	19.7	15.3
19:1	0.2	0.3	0.1
20:1	4.8	3.8	10.5
22:1	4.9	4.3	13.1
24:1	0.3	0.2	0.5
Σ	45.8	43.7	52.3
Polyunsaturated			
16:2ω4	0.5	0.5	0.4
16:3ω4	0.4	0.3	0.4
16:4ω1	0.4	0.5	0.6
18:2ω6	0.8	1.0	1.3
18:3ω6	0.4	0.5	Trace
18:3ω3	0.6	0.8	0.4
18:4ω3	0.9	1.0	1.3
20:2ω6	0.2	0.4	0.3
20:3ω6	NSA	NSA	0.1
20:4ω6	1.3	1.7	0.6
20:4ω3	0.7	0.7	0.5
20:5ω3	15.3	17.4	9.3
21:5ω2 ?	0.5	0.6	0.2
22:2ω6	0.1	0.2	NSA
22:4ω6	0.4	0.3	0.4
22:5ω6	0.3	0.2	0.2
22:5ω3	0.9	1.3	0.7
22:6ω3	8.9	8.6	8.1
Σ	32.6	36.0	24.7

[a]NSA = no significant amount.
[b]The ω9 isomers should predominate, but others may be present.

The analyses of samples A and B are in agreement in most respects. It is possible that the slightly lower levels of 20:1 and 22:1 and the slightly higher level of 20:5ω3 in sample B are associated with the roe lipids. Whole cod roe lipids, for example, contain very little of these two monounsaturated fatty

acids (Ackman and Burgher, 1964), and rock lobster (*Jasus lalandii*) roe contains phospholipids (50% of the lipid of the roe) in which the ratio of 20:5ω3 to 22:6ω3 is 2.5:1 (de Koning and McMullan, 1966). Lipids constitute 12% of the weight of rock lobster roe and 15% of the roe of the freshwater crayfish *Orconectes rusticus* (Wolfe, 1964). Female mysids (*Neomysis integer*) contain slightly more lipid than males but it is not clear if this is due to the larger average size of the females or to the presence of the lipid-rich eggs (Linford, 1965). Eggs also raise the lipid level seasonally in gravid females of the copepod *Euchaeta antarctica*, but not in the euphausid *Euphausia crystallorophias* (Littlepage, 1964). This relates to whether the young possess a large yolk sac or have food immediately available on hatching.

The lipids of North Pacific zooplanktonic crustacea are characterized by generally high iodine values ($\sim$ 200) and high levels of non-saponifiable material (10–50%) (Yamada, 1964). The meagre detailed fatty acid data available from this area and Antarctica (Ackman and Eaton, 1966) and the iodine values suggest the virtual absence of 20:1 and 22:1 in most zooplanktonic crustacea, in sharp contrast to the results for *M. norvegica*. Analyses of mixed zooplankton samples from the North Atlantic (Klenk and Eberhagen, 1962; Brockerhoff et al., 1964b) also show very low overall levels of 20:1 and 22:1 suggesting that this observation is not associated with any specific ocean.

The particular sample of *P. borealis* is intermediate between *M. norvegica* and other zooplankton in lipid properties and fatty acid composition. The fatty acid recovery and composition suggests that the lipids are similar to those of the lobster (*Homarus americanus*). Brockerhoff et al. (1964a) recovered 1.91% lipids from a whole lobster of which only 60% was triglycerides. Planktonic crustacea are generally subject to wide variations in lipid content (Fisher et al., 1954; Fisher, 1962), although the fall months are usually those in which stores of depot fat are highest.

The fatty acid compositions of phospholipids and triglycerides are dissimilar in most marine organisms, particularly in 20:1 and 22:1, and the overall fatty acid compositions of different organisms not feeding directly on phytoplankton therefore reflect the proportions of type of lipid. In mixed zooplankton from Nova Scotia waters (late March) Brockerhoff et al. (1964b) found that triglycerides (8% of the total lipid) contained modest levels of 20:1 and 22:1 although the phospholipids (54% of the total lipids) had only trace amounts of these acids.

Fisher (1962) mentions that the fatty acid compositions of *M. norvegica* and *Thysanoessa raschii* (from the same area, Loch Fyne, and presumably taken at the same time of year) had markedly different compositions, but the depot fat accumulations in these two species do not take place simultaneously (Fisher et al., 1954).

Both Linford (1965) and Littlepage (1964) draw attention to the fact that not all zooplankton are subject to similar seasonal fluctuations in lipid. In some species lipid reserves are obviously catabolized during periods when

food is scarce, in others the metabolism becomes sluggish to tide the animals over this period. Fat is probably still the most important energy source under the latter conditions (Conover, 1962).

Despite the interest in the metabolism of zooplankton we are unaware of any detailed study of the types of lipid in relation to seasonal variations. These are required to clarify the question of whether *M. norvegica* has a unique fatty acid composition or whether the sample analysed happened to be exceptionally rich in triglycerides.

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

R. G. ACKMAN
C. A. EATON

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Three shrimp taken in late January 1967, and held alive 3 weeks without food were killed and immediately examined for lipid as described above. Lipid recovery was 0.83% and chromatographic separation monitored by thin-layer chromatography showed the lipid to be approximatly 47% phospholipid, 43% triglyceride, and 10% sterol with a small amount of diglyceride. Free fatty acids and sterol esters were not observed. Pigment was estimated spectrophotometrically at $<0.1\%$.

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Guide to Contributors

Journal of the Fisheries Research Board of Canada

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STYLE AND ORGANIZATION

Original research papers should conform as far as possible to the following pattern, although unnecessary standardization is not desired:

Title must be kept short, specific, and informative for retrieval systems. Subtitles and series papers should be avoided.

Abstract must be complete in itself. It should be informative in scope, methods, and results; briefly indicate significance of the findings; be free of unnecessary description; and not exceed 200 words. Authors are encouraged to provide an abstract in both languages; one must be provided in the same language as the text.

Introduction should outline the problem, referring only to pertinent investigations, and define the objectives or reasons for the study.

Materials and methods or *Experimental procedures* should include a description of design and organization of the investigation. Procedures, including those for statistical analyses of data, must be either referenced or described in enough detail to permit the work to be repeated.

Results are preferably separated from discussion. The text must be meaningful without reading of tables, illustrations, or other research; these should be referred to in parentheses. Only data necessary to support the text should be given. Data must not be duplicated in tables and in illustrations, and should be summarized so that they can be easily understood. Statistical analyses should be used when appropriate to aid in interpreting the data, but should not mask the comparisons of interest. Inclusion of preliminary or raw data, even in an appendix, is discouraged. Measurements should be reported in metric units.

Discussion should give the significance of the findings, and their relation to published research. Importance of this section cannot be overemphasized.

Conclusions as a separate section are optional.

Acknowledgments, if included, are placed at end of text.

References should be listed alphabetically by author, without numbers, in the form described by the *Style Manual for Biological Journals*. Citations in the text are made by the *name-and-year* system. Manuscript reports may be listed in the References, but should be cited in the text with the letters "MS" preceding the year.

Notes are for publishing research with narrow boundaries that may result as a by-product of investigations, for describing research techniques and fishing methods, for establishing publication priority, and for reporting useful information from projects that were terminated before completion. This section is not a repository for inferior research. If the scope of the Note permits,

it may be organized like a research paper except that it must have no abstract and the author is identified at the end. Total length must not exceed four printed pages. Otherwise, organization is flexible.

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